Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

A99.9
F769
C2

PROCEEDINGS

19th SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

June 16-18, 1987

College Station, Texas

USDA
NAT'L. AGRIC. LIBRARY
APR 18 P 8:30
CURRENT SERIAL RECORDS

Sponsored by:
The Southern Forest Tree Improvement Committee,

The Texas Forest Service

and

The Texas Agricultural Experiment Station

PROCEEDINGS OF THE

NINETEENTH SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

June 16-18, 1987

College Station, Texas

Sponsored Publication No. 41 of the

Southern Forest Tree Improvement Committee

PREFACE

The Nineteenth Southern Forest Tree Improvement Conference was held at the College Station Hilton, near the Texas A&M campus in College Station, Texas. The Conference was co-sponsored by the Texas Forest Service and the Texas Agricultural Experiment Station.

Following the tradition established at the eighteenth SFTIC Conference, the non-invited papers were judged by the SFTIC Committee members based on the oral presentations as candidates for the Tony Squillace award of $200. Four nominees were chosen by SFTIC. The final selection was made by the program committee bases on the written presentations. We are pleased to announce that the winning paper is: "Annual Top Pruning as a Crown Management Technique in a Young Loblolly Pine Seed Orchard to Reduce Height and Still Produce Flowers" by D. M. Gerwig. Congratulations to Mr. Gerwig.

19th SFTIC Program Committee: C. R. McKinley
W. J. Lowe
J. P. van Buijtenen

The papers in these proceedings have been published as they were submitted by the authors--in camera-ready form. Responsibility for the technical content remains with the respective authors.

Copies of this publication can be obtained by writing to:

The National Technical Information Service
Springfield, VA 22161

C O N T E N T S

KEYNOTE ADDRESS Page

A Challenge for Tree Improvement
W. T. Gladstone, P. M. Bean, J. H. Hughes and C.B. Talbert 1

GENERAL SESSION

Genetic Improvement of Virginia Pine Christmas Trees
G. F. Brown, Jr. 9

A Tree Improvement Program to Develop Clones of Loblolly Pine for Reforestation
G. S. Foster and D. V. Shaw 17

Area Potentially Available to a Tree: A Research Tool
W. R. Smith 22

Dynamics of Stand Growth and Yield Over 29 Years in a Loblolly Pine Source Trial in Arkansas
C. B. Talbert and M. R. Strub 30

Polymix Crosses for Rust Resistance Screening
T. D. Byram, W. J. Lowe and C. R. McKinley 39

Regeneration of Yellow-Poplar from Protoplast Culture
S. A. Merkle and H. E. Sommer 45

The Nature of Waterlogging Tolerance of Loblolly Pine
D. D. Hook and T. Shear 51

BIOTECHNOLOGY I

Applications of Biotechnology in Forest Tree Improvement (invited paper)
J. W. Hanover 59

Cellular Drought Tolerance Studies in Loblolly Pine
R. J. Newton, J. D. Puryear and S. Sen 71

Chloroplast Ultrastructure of Primary Needles in the Adventitious Shoots of Pinus taeda
J. S. Yi and H. E. Sommer 79

The Frequency of Plant Regeneration from Norway Spruce Somatic Embryos
M. R. Becwar, S. A. Verhagen and S. R. Wann 92

Page

Stage of Shoot Development and Concentration of Applied Hormone Affect Rooting of Northern Red Oak Softwood Cuttings
R. M. Teclaw and J. G. Isebrands 101

BIOTECHNOLOGY II

Osmotic Adjustment in Pinus taeda Families in Response to Water Stress
C. E. Meier and R. J. Newton 110

Growth and Isozyme Analysis of Axenic Cultures of Cronartium quercuum f. sp. fusiforme Derived from Basidiospores
S. F. Shamoun, F. H. Huang and D. A. Slack 118

Chloroplast DNA Variation in the Sympatric Zone of Lodgepole pine (Pinus contorta Dougl.) and Jack Pine (P. banksiana Lamb.)
D. B. Wagner, D. R. Govindaraju and B. P. Dancik 119

The Tendency of Round Gall Development in Loblolly Pines that are Resistant to Cronartium quercuum f. sp. fusiforme
G. A. Snow 124

Seasonal Rooting Response of 6-Year-Old Loblolly Pine Clones to Different Concentrations of Indole-3-Butyric Acid and Dimethyl Sulfoxide
M. F. Mahalovich, S. E. McKeand and J. B. Jett 125

Pitch Canker Resistant Slash Pine Identified by Greenhouse Screening
S. W. Oak, G. M. Blakeslee and D. L. Rockwood 132

Silicon Effects on Loblolly Pine Seedling Growth and Water Status
S. F. Emadian and R. J. Newton 140

Use of Excessive Waterlogging and Physiological Responses to Measure Genetic Variation in Loblolly Pine Waterlogging Tolerance
T. H. Shear and D. D. Hook 146

Induction of New Host Coded Proteins in Pinus elliottii Seedlings in Response to Pathogen and Water Stres
J. V. Valluri, E. J. Soltes, R. J. Newton and B. G. Cobb 152

Page

SEED ORCHARD MANAGEMENT I

Seed Orchard Management: Something Old and Something New (invited paper)
J. B. Jett 160

Value of Production Orchards Based on Two Cycles of Breeding and Testing
C. G. Williams and J. E. de Steiguer 172

Potential Alternative Insecticides for Insect Control in Southern Pine Seed Orchards
R. S. Cameron, G. L. DeBarr, J. F. Godbee and J. W. Taylor 182

Seed Yields per Bushel: Orchard vs. Natural Stands
K. O. Summerville 190

The Weiss-McNair Cone Harvester
G. A. Lowerts and M. H. Zoerb, Jr. 195

SEED ORCHARD MANAGEMENT II

Phenotypic Selection Effective in a Northern Red Oak Seedling Seed Orchard
T. LaFarge and R. A. Lewis 200

Annual Top Pruning as a Crown Management Technique in a Young Loblolly Pine Seed Orchard to Reduce Height and Still Produce Flowers
D. M. Gerwig 208

Supplemental Mass Pollination is Feasible on an Operational Scale
F. E. Bridgwater, D. L. Bramlett and F. R. Matthews 216

Clonal Variation of Tannin and Phenolic Concentrations of Cones from a Selected Population of Loblolly Pine
T. N. Trembath and G. R. Askew 223

The Effects of Using Pollen Contaminated with Conidia of Fusarium moniliforme var. subglutinans on Control-Pollinated Strobili of Slash Pine
T. Miller, G. M. Blakeslee, D. L. Bramlett and F. R. Matthews 232

An Operational Trial of Supplemental Mass Pollination in a Loblolly Pine Seed Orchard
T. D. Blush 240

Page

A Southern Pine Seed Orchard in the Lower Rio Grande Valley of Texas
C. R. McKinley 247

Effect of a Clonal Row Orchard Design on the Seed Yields of Loblolly Pine
D. L. Bramlett and F. E. Bridgwater 253

Design, Establishment and Management of a Black Alder (Alnus glutinosa L. Gaertn.) Seed Orchard
R. B. Hall and R. N. Nyong'o 261

Effects of Related and Unrelated Graft Partners in Slash Pine (Pinus elliottii Engelm.)
R. C. Bower and C. R. McKinley 269

DESIGN AND MANAGEMENT OF PROGENY TESTS

Practical Uses of Breeding Values in Tree Improvement Programs and Their Prediction from Progeny Test Data (invited paper)
T. L. White and G. R. Hodge 276

Relationship Between Stability Indices at the Time of Germination and at the Seedling Stage in Jack Pine
D. R. Govindaraju and B. P. Dancik 284

Field and Greenhouse Fusiform Rust Symptoms Predict Mortality in Progeny Field Tests
C. H. Walkinshaw 292

APA: A Useful Tool for Analyses of Progeny Tests
S. B. Land, Jr. and W. L. Nance 300

Regression and Spline Methods for Removing Environmental Variance in Progeny Tests
B. C. Bongarten and J. F. Dowd 312

Progeny Testing Nitrogen Fixing Trees Prosopis (Mesquite) and Leucaena for Biomass Production
P. Felker 320

A Seed Source and Progeny Test of Select East Coast Loblolly Pines in Arkansas and Mississippi
R. C. Schmidtling 329

GROWTH AND YIELD OF GENETICALLY IMPROVED MATERIAL

Effects of Genetically Improved Stands on Growth and Yield Principles (invited paper)
P. T. Sprinz 338

Page

Patterns of Genetic Variation Among Ten-Year-Old Open-Pollinated Mid-South Seed Sources of American Sycamore
J. R. Toliver and S. G. Dicke 349

Geographic Genetic Variation in Black Cherry
C. S. P. Patil and S. B. Land, Jr. 357

Modeling Distributions of Stem Characteristics of Genetically Improved Loblolly Pine
J. E. Janssen and P. T. Sprinz 367

Juvenile-Mature Correlations in Sycamore
E. B. Schultz and S. B. Land, Jr. 376

Isozyme Heterozygosity, Phenotypic Family Stability, and Inter-Family Competition Among Seedlings of Loblolly Pine
G. A. Tuskan and A. E. Wiselogel 384

Results of Micropulping Wood Samples of Seven Pine Hybrid Families in Zululand
J. A. Wright 399

Differences in Growth Rate and in Acceleration of Growth Rate Among Loblolly Pine Rangewide Seed Sources Confirmed
F. H. Kung 407

Geographic Genetic Variation in Southern Red Oak
A. M. Mukewar and S. B. Land, Jr. 415

Tests of Realized Genetic Gain from a Coastal Virginia Loblolly Pine First Generation Seed Orchard
G. A. Lowerts 423

APPENDIX

Addendum: Views on Forest Biotechnology: Report from a Conference on Tree Improvement by Genetic Engineering 433

List of Attendees, 19th SFTIC 444

SFTIC Membership 453

A CHALLENGE FOR TREE IMPROVEMENT

W.T. Gladstone[1], P.M. Bean[2], J.H. Hughes[1], C.B. Talbert[1]

Abstract.--Growth gains have been projected from several levels of genetic testing. Hard evidence that expected gains are being realized on an operational scale is scarce, and its acquisition will be difficult. Evidence from every potential source should be examined for direct or inferential bearing on the question. Yield trial, progeny test, and field verification information are discussed herein as exemplary sources, and a positive case is made for substantial growth gains in a specific circumstance. The tree improvement community in the South has a collective responsibility to demonstrate that its achievements are real and implementable, and it should be appropriate to meet that responsibility through collective action. Our clients are asking the questions. When will we have the answers?

Additional keywords: loblolly pine, family plantations, progeny tests, yield trials, realized gain.

Maybe the Georgians were right, back in 1983[3], when they suggested that actual realized gain, at least on an operational scale, from our first generation of tree improvement may never be known. No one is eager to plant much woods-run comparison acreage for the sake of estimating that phantom factor, and that fact in itself is a good indication of a strong general preference for improved stock. But that preference, in turn, is sometimes motivated less by confidence and conviction than by indoctrination and fear of the unknown, sort of like the wayward soul who daresn't blaspheme, just in case. At any rate, the frequency with which questions about how we are really doing and how our orchard programs are paying off are being asked is on the rise, and we, as a southern tree improvement community, have a responsibility to respond with the very best answers we can muster.

The short term answers may all be inferential, by necessity, but they must be as strong and convincing as we can make them. In the long term, it would be prudent to develop some genetic standards which are precisely reproducible and which, because they are also good producers, can be deployed on sufficient acreage to provide some bases for direct measurement of improvement progress (e.g., full sib families, clones?). However we

---

[1] Southern Forestry Research, Weyerhaeuser Company.

[2] North Carolina State Forest Nutrition Cooperative.

[3] Tankersley, L., B. Bongarten, G. Brister, and M. Zoerb. 1983. Operational plantations of improved slash pine: Age 15 results. 17th South. For. Tree Improv. Conf. Proc. Athens, GA. p. 271-280.

approach it, it's important that an effort is made in every program to accumulate inferential evidence now, and direct evidence later, as we develop and use more advanced material. One more modest contribution to the relatively small stockpile of large scale gain evidence follows.

Plantation Verification

In 1986, we established the mean exhibited site indices for 101 single family plantations in North Carolina. These loblolly stands ranged from five to seven years of field age; represented 16 percent of the plantations we established in 1979, 1980, and 1981 in that region; and were stocked with seven of our best half-sib families. These same seven families are growing on 91 percent of our 1979-81 North Carolina plantations. As indicated in Table 1, mean site indices for these families, as exhibited at ages 5-7, ranged from 68 to 74 feet. Particularly gratifying to us was confirmation that we can discriminate among families with respect to growth even when they are arrayed across 6200 acres of operational plantations. This suggests that remeasurement over time and the scale use of genetic standards might be useful strategies in establishing the worth of orchard lines. While relative growth rates among genetic entities in the field proved to be measurable, the usual dearth of wood-run plantations forced us to turn our attention next to other sources of information, e.g. progeny tests and yield trials, to try to get a handle on the phantom factor.

Table 1 -- Site index means by family for 1979, 1980, and 1981 plantations in North Carolina, as exhibited in 1986.

Family	Number of Plots	Mean site index (feet)	Waller-Duncan T-Test (.05)
74	183	74.3	A
68	212	73.6	AB
42	165	72.9	BC
01	165	72.5	BC
103	142	72.1	C
76	149	69.6	D
59	170	67.9	E

Progeny Test

To that end, we remeasured a 22 year old full-sib progeny test of loblolly pine which had four clonal parents in common with the families which were field verified. This early test was limited in the number of crosses representing most clonal parents, however those parents whose progenies performed unusually well in this test have been eliminated from the program by the weight of cumulative testing, i.e., 78, 63, and 46 didn't hold up across other progeny tests (Table 2). This 1964 wet-site test was not bedded, was not intensively managed, and had conventional ten-tree row plots with 8 by 9 foot spacing. Only the three fertilized

replications were measured and at age 22, had stocking of 480 tpa. (79 percent survival), basal area of 180 ft^2/a., exhibited site index of 68 ft., and standing volume of 4560 ft^3/a.

Table 2 -- Family (clonal) means for volume per tree at age 22, from a 1964 full-sib progeny test. This test was not intensively managed.

Family	Cubic Feet Per Tree	Crosses Per Family	Family Still In Program?
78	11.6	1	No
74*#	11.6	1	
76*#	11.1	2	
102 #	11.1	3	
68*	11.0	6	
141	10.4	1	
63	10.1	1	No
46	9.8	2	No
21 #	9.7	2	
103*	9.5	1	
131	9.5	1	
31	9.3	3	
64	9.0	2	No
73	8.9	2	
53	8.9	3	No
Check	8.8	-	No
33	8.4	9	No
65	7.7	1	No
142	7.5	1	No

* Represented in field verification.
# Compared with yield trial results.

The mean tree volume of the collected families exceeded that of the check trees by 8 percent, as did the corresponding survival rate. Assuming an extrapolated stand density of 444 tpa for the check trees, 45.6 cunits per acre (actual) and 39.1 cunits per acre (extrapolated) respectively represent the standing volumes of the family group and the check trees, or a gain over check of 17 percent (Table 3). Considering the risks of expanding intermixed row plots into a homogeneous stand, the 39.1 cunits per acre check volume can be considered a minimum estimate of what a pure stand of check trees might have produced.

To relate the progeny test information more closely to the field verification, we next compared the performance, relative to check in the progeny test, of the four families common to both efforts (Tables 2, 4). Note that while the proportion of all test trees that were from these four families was 34 percent, their contribution to total test volume was 43

Table 3 -- Comparison of all families with hypothetical check block; 1964 full-sib progeny test, age 22.

	All Families	Check Trees	Gain
Mean volume/tree (ft^3)	9.5	8.8	8%
Survival rate (tpa)	480 actual	444 extrap.	8%
Total volume (ft^3/acre)	4560 actual	3910 extrap. (lower bound)	17%

percent. Mean volume per tree as measured in the four families beat that of the check trees by 23 percent. A 100 tpa or 23 percent advantage in extrapolated survival for the four families produced extrapolated volumes of 58.8 and 39.1 cunits per acre for the four families and the checks, respectively, or a gain estimate of 50 percent. Again, recognizing that we have established ideal upper and lower bounds, the real gain probably lies between 23 and 50 percent for the four families which were evaluated in the field verification project.

Table 4 -- Comparison of families 68, 74, 76, and 103 with hypothetical check block; 1964 full-sib progeny test, age 22.

	Four Families	Check Trees	Gain
Mean volume/tree (ft^3)	10.8	8.8	23%
Survival rate (tpa)	544 extrap.	444 extrap.	23%
Total volume (ft^3/acre)	5880 extrap. (upper bound)	3910 extrap. (lower bound)	50%

Proportion of all test trees from families 68, 74, 76, 103 = 34%.
Proportion of total test volume from families 68, 74, 76, 103 = 43%.

Family Yield Trial

In the spring of 1978, we established a yield trial which would permit comparisons with woods-run check of 16 North Carolina half-sib families when grown in pure family blocks and in blocks which contained non-contiguous mixtures of the same 16 families. Pure families, checks, and mixtures were all grown in 100 tree rectangular plots at 7 by 9 foot spacing, with all measurements confined to an internal 64 trees on each plot. This study was intensively managed and tree heights averaged 28 feet at 8 years from planting, an overall exhibited site index of about 79 feet.

The productivity of the collective pure family blocks was significantly higher than the check block, with superiority in height, survival, and volume per tree contributing to a significant 16 percent gain over check in total volume per acre (Table 5). The mixed families did not do as well, producing lower gain values in general, including an 11 percent gain in total volume per acre, which was not statistically significant. The apparently better performance of the families in pure blocks should provide reinforcement to those of us who live by the family and perhaps some incentive to those who do not. We view this evidence as analogous to the superiority of monoclonal over multiclonal eucalyptus management, as it has been so clearly demonstrated by the Brazilians. We'll follow the growth of this study with interest and bated breath, and doubtless will establish others with advanced material.

Table 5 -- Mean superiority over check for 16 half-sib entries in the family yield trial, at age 8.

Plot Type	Superiority over check for: Height %	DBH %	% Survival %	Vol/Tree %	Vol/Acre %
Pure family	5.8****	1.7ns	6.8***	9.0*	15.6**
Mixed family	3.8**	0.7ns	6.8**	5.1ns	11.3ns

** Significant at 0.01 level.

Yield Trial - Progeny Test Comparison

Mean tree volumes for common families in the yield trial compare well in rank order with those in the progeny test (Table 6) particularly if family 78 is ignored.

Table 6 -- Comparisons of mean tree volumes for families common to the full-sib progeny test (age 22) and the half-sib yield trial (age 8).

Family	Volume Per Tree: Progeny Test	Yield Trial	Family Still in Program?
	ft^3	ft^3	
78	11.6	1.7	No
74	11.6	2.0	
76	11.1	2.0	
102	11.1	1.8	
21	9.7	1.8	
31	9.3	1.7	
Check	8.8	1.6	No
33	8.4	1.6	No

Mean superiorities over check for all families (pooled), as calculated from pure blocks in the yield trial, were virtually identical with those of the progeny test, when considered for volume per tree, percent survival, and volume per acre (Table 7). Both tests indicate a gain in volume per acre of 16-17 percent.

Table 7 -- Mean superiority over check for all families, as calculated from pure blocks in the family yield trial and from the 1964 progeny test.

Study	Superiority Over Check for: Volume/Tree	% Survival	Volume/Acre
Yield Trial (8 yrs)	9%	7%	16%
Progeny Test (22 yrs)	8%	8%	17%

Families 21, 74, 76, and 102 were common to the yield trial and progeny test, and when pooled within each test, yielded gains over checks of 20 and 24 percent, respectively, in volume per tree (Table 8). In both studies better family survival (better than in check lots) pushed the per acre volume gains higher, to 27 percent in the yield trial and to 41 percent in the progeny test.

Table 8 -- Mean superiority over check for families 21, 74, 76, and 102, as calculated from pure blocks in the family yield trial and from the 1964 progeny test.

Study	Superiority Over Check for: Volume/Tree	% Survival	Volume/Acre
Yield Trial (8 yrs)	20%	5%	27%
Progeny Test (22 yrs)	24%	14%	41%

Yield Trial - Verification Inferences

The eight year old family yield trial also had four families in common with the plantation verification effort, namely 1, 59, 74, and 76. As shown in Table 9, within the yield trial, the pooling of these four families produced gains over check of 19 percent, 5 percent, and 26 percent, respectively, in volume per tree, survival, and volume per acre. Recalling that we established a 23 percent gain in volume per tree for the four family set in the progeny test and that we concluded that the volume per acre gain in that test probably lies between 23 and 50 percent, the best evidence that we can muster at present implies that our 1979-81 plantations are producing and will continue to produce 25 percent more wood than they would have had we not gotten into the tree improvement business. Six of the seven families which stock 91 percent of this period's

plantations are represented in one or both of the tests upon which we base this inference.

Table 9 -- Comparison of families 1, 59, 74, and 76 with check blocks, family yield trial, age 8.

	Four Families	Check Blocks	Gain
Mean Volume/Tree (ft^3)	1.9	1.6	19%
Survival Rate (tpa)	639	608	5%
Total Volume (ft^3/acre)	1210	970	26%

Conclusion

Data from a variety of sources can contribute to inferential assessment of growth gains. The ability to track specific genetic entities over time and across substantial plantation acreages can enhance that assessment. Industries, agencies, and universities supporting tree improvement programs have a vested interest in (1) securing and publicizing current yield information; (2) establishing trials, side-by-side comparisons, etc., which will better equip us to answer questions about the oncoming generations of material; and (3) considering the joint or independent development of some highly productive, reproducible genetic standards. The phantom factor will succumb only to thought, sweat, and dollars, but a great deal of cooperation among the players can minimize these inputs.

GENERAL SESSION

MODERATED BY DR. J. C. LEE

Texas A&M University

GENETIC IMPROVEMENT OF VIRGINIA PINE CHRISTMAS TREES

G. F. Brown Jr. [1/]
Assistant Professor
Department of Plant and Soil Science
Alabama A&M University, Normal, AL 35762

Abstract.-- Approximately 10 half-sib families of Virginia pine from 9 different sources located in Alabama, Mississippi, South Carolina and Tennessee were grown and evaluated after 3 years in a test in north Alabama. Characteristics measured pertinent to Christmas tree production were height, diameter, branches per whorl, needle color, stem straightness and an overall quality rating. There were significant differences among sources, however an extreme amount of variation due to families-within-sources and among trees within each plot was also observed. Estimates of narrow-sense heritabilities ranged from a low of $h^2 = 0.10$ for branches per whorl to a high of $h^2 = 0.35$ for needle color. Stem straightness and height were found to be the most important characteristics for an improvement program. Potential genetic gains are presented with selection emphasizing the above two characteristics. Results of this study were compared to the results obtained in 3 similar tests demonstrating the effect of site quality on estimates of heritabilities and genetic gains.

Additional keywords: Pinus virginiana, heritability, genetic gain, provenance test.

In the 1960's and 1970's Christmas tree growers from several southern states organized growers' associations, signifying the establishment of the industry in the South. The primary species being utilized was Virginia pine (Pinus virginiana) (Davis et al. 1981; Hu and Main 1980). Over 10 million Virginia pines were planted across the south in the 1982-83 planting season (Murray, 1983). It is evident that the Virginia pine has been accepted by consumers and growers and has become the foundation species for the Christmas tree industry in the South.

The key to a successful Christmas tree operation is the production of high-quality trees. The quality of the trees is largely dependent upon the cultural practices used by the grower and the genetic potential of the tree. While appropriate cultural practices were established early, little has been done to improve the genetic component. Seedlings being used to establish Christmas tree plantations are grown from seed collected from wild stands or from parents in seed orchards originally selected for pulpwood characteristics. To compound the problem, a large portion of the seedlings (possibly between 30 and 50 percent) planted throughout the South have originated from one orchard comprised of eight clones, all selected

---

[1/] Assistant Professor, Alabama A&M University, Normal, Alabama.

from one county in Alabama.

Estimates of heritabilities are necessary before an effective breeding program can be initiated. They also are used to predict the amount of genetic gain that can be anticipated from the improvement program. The purpose of this study is to give estimates of heritabilities ("narrow sense") and the potential genetic gains they indicate for several characteristics of importance in Virginia pine Christmas tree production.

METHODS

During the fall of 1980, members of the S-128 Regional Research Project collected open-pollinated seed from 16 sources of Virginia pine. Seedlings from 8 of these collections, 2 from Alabama, 3 from South Carolina, 2 from Tennessee and 1 from Mississippi along with 11 half-sib families from the Kimberly-Clark Corporation seed orchard were used to establish a progeny test at Alabama A&M University during the spring of 1983. The 85 half-sib families representing the above 9 sources were planted in a randomized complete block design with six-tree-row family plots and 6 replications. Trees were planted 4 feet apart within each row and 7 feet between rows. One border row was planted around the entire test.

The planting site was mold-board plowed and disked prior to planting. Weeds were controlled within each row by use of herbicides and between rows by mowing. Several insecticide sprayings were made to control the Nantucket pine tip-moth. The trees were allowed to grow naturally for 3 years and were not sheared.

Each tree was evaluated for total height, stem diameter, crookedness, needle color, branches per whorl, and an overall quality rating. The evaluation of crook and rating were based on a five-point scale with 5 as the highest score given to the straightest or best trees.

The estimate of variation for sources and families-within-sources were obtained using both the General Linear Model (GLM) and Variance Component procedures of Statitistical Analysis Systems (SAS) (SAS Institute 1979). Estimates of narrow-sense heritabilities were then calculated . Source differences were then compared to results obtained from the Alabama A&M planting of the S-128 seed source study. Heritability estimates of this study (A&M -FWS) were compared to the results obtained in 2 previous half-sib progeny tests conducted by the author at Alabama A&M (A&M) and at the University of Tennessee Highland Rim Field Station (HRFS) and a similar progeny test conducted at Auburn University (Aub) (Warlick et.al. 1985). The average of all estimates of heritabilities was used to calculate potential genetic gains. This resulted in an estimate of genetic gain based on at least 7,000 trees from 200 half-sib families.

RESULTS AND DISCUSSION

Seed Source Differences

Two of the most important characteristics evaluated were height and crookedness. The means for these characteristics for the 9 sources are

presented in Table 1. The means for these characteristics obtained in the A&M planting of the S-128 seed source study are also presented for comparison purposes.

TABLE 1. -- Seed source means for height and crook of Virginia Pine at 3 years.

Source	Height (cm)		Crook (5-pt. scale)	
	A&M (FWS)	A&M (S-128)	A&M (FWS)	A&M (S-128)
K-C	170.4 [a]		3.54 [ab]	
Telico, TN.	161.1 [b]	189.7 [a]	3.39 [bc]	2.73 [a]
Green Mt., AL.	160.7 [b]	183.0 [abc]	3.53 [ab]	3.00 [a]
S.C. Up. Pied.	159.0 [b]	188.8 [ab]	3.32 [c]	2.69 [a]
Anderson Co., TN.	157.8 [b]	177.3 [abc]	3.09 [d]	2.91 [a]
S.C. Mid. Pied	157.0 [bc]	193.7 [a]	3.30 [c]	3.35 [a]
S.C. Low. Pied	155.4 [bcd]	170.8 [bc]	3.38 [bc]	2.71 [a]
Tish.Co., MS.	151.6 [cd]	168.0 [c]	3.31 [c]	2.54 [a]
Dekalb Co., AL.	149.8 [d]	191.1 [a]	3.69 [a]	3.29 [a]

Means within each column followed by the same letter do not differ at the 0.05 level of significance according to the Duncan's Multiple Range Test.

The Kimberly-Clark Corporation orchard seedlings were significantly taller and ranked second in stem straightness. These results are expected because of the comparison of select seed orchard material versus seed collected from natural stands. There was only an 11 cm difference in height from the next tallest source to the shortest source. Also, the Dekalb Co., AL. source which was the shortest in the FWS study was the tallest in the S-128 study. This source also had the best score on stem straightness. Although the source differences were highly significant, it never accounted for more than 2.5 percent of the total variation observed in any of the characteristics which were evaluated. For these reasons, seed source differences are not considered to be very important in a breeding program for the improvement of Virginia pines for Christmas tree production in north Alabama. However, it should be noted that none of the seed sources tested originated more than 200 miles north of the test location.

Heritabilities

The estimates of narrow-sense heritabilities are presented in Table 2 for the results of this study, two other studies completed by the author and one study completed at Auburn University. The highest heritability obtained in all studies was for needle color, while the lowest was generally for branches per whorl. The heritability estimates also varied according to study location. The HRFS location yielded the best growth and lowest heritability for height. The A&M location yielded the poorest growth and highest height heritability primarily because of a heavy compacted soil. This study (FWS) was located next to the A&M study, but was mold-board plowed prior to planting, yielding much better growth and a lower heritability estimate. A higher heritability can be the result of either or both of the following: (1) the denominator of the equation, primarily the component due to the environmental variance, may be smaller, and/or (2) the additive genetic component, families-within-source, is larger. In these studies the relative amount of environmental variance, primarily the within-plot variance component, was lowest at the HRFS location and highest at the A&M location. This suggests that the better site benefits the poorer trees relatively more than the better trees, resulting in less tree-to-tree variation within a plot.

TABLE 1. -- Estimates of heritabilities of three-year-old Virginia pine from 4 half-sib progeny tests.

Variables	Progeny Test A&M	HRFS	A&M (FWS)	Auburn	Average
Height	0.28	0.13	0.19	0.16	0.19
Diameter	0.16	0.15	0.16	0.13	0.15
Crook	0.12	0.19	0.14		0.15
Color	0.39	0.38	0.35		0.37
Branches	0.16	0.11	0.10	0.12	0.12
Rating	0.15	0.15	0.19		0.16

The above heritability estimates are fairly low for most of the variables. This is due primarily to a large within-plot variance component which accounted for between 42 and 61 precent of the total phenotypic variance. The second largest source of variation in all cases was the component due to the interaction of replications and families-within-source. These two components accounted for between 73 and 90 percent of the total variance for all characteristics. The tremendous amount of tree-to-tree variation within a family presents a large obstacle to the

genetic improvement of Virginia pine Christmas trees by lowering the estimates of heritabilities and potential genetic gains.

Selection strategy

As previously stated, the first level of selection - selection of the best seed sources - is not of primary of importance. The next level is selection of the best families within each source. Previous research by the author (Brown 1987) has shown that there are no significant interactions between families and locations in north Alabama and central Tennessee. The best families can be used in a breeding program for the areas tested negating the need to make separate selections and establish different seed orchards.

The next selection level is the selection of individuals within families. However, the selection of families and individuals within families brings up an important question: What characteristics should be emphasized in a breeding program? A quality Christmas tree is a combination of several characteristics, such as good growth, dense crown, straight stem, and dark needle color. To maximize one characteristics would likely be at the expense of other characteristics. This could result in an overall decrease in tree quality or performance.

Previous research has eliminated the characteristics of internodal distance, limb angle and crown form, because of extremely low heritabilities, lack of significant differences among families-within-sources or high correlations with other more important characteristics. For example, stem diameter can also be eliminated for similar reasons. The best Christmas tree would be the tallest with the smallest stem diameter. However, there is a positive correlation between height and diameter of 0.81. Selection for a smaller stem diameter may result in a negative effect on height.

In an attempt to determine which characteristics have the greatest influence on overall tree quality, a multiple regression analysis was performed using the rating as the dependent variable being predicted by the other characteristics. The most signifiant characteristic accounting for more than 37 times the amount of variation of the second factor, was the crook value. To verify the magnitude of importance of this characteristic, 5 experienced Christmas tree growers and field workers remeasured and evaluated one replication. The result of the regression analysis of this data was similar, in that crook was the predominant predictor of overall tree quality. A shorter or "open" tree can still be a merchantable product, whereas a crooked tree, regardless of its height or density is commonly not salable. Based on these results and several other factors, it appears that the selection of superior trees should be most heavily weighted by the "quality rating" characteristic and stem straightness, followed by height growth with the least emphasis being placed on branches per whorl and needle color.

A commonly used method of selecting families for more than one trait is to remove all families not significantly better than the family with the worst performance. However, because of the large amount of variation in the error term for families-within-sources, using this method on the three most important characteristics eliminates over 90 percent of all families.

This culling is too drastic to maintain the genetic diversity needed for a breeding program.

The method utilized in this study was based on the calculation of the standard error of the means for each characteristic. Those families which were more than one standard error below the overall mean for two or more characteristics were culled. All families more than two standard errors less than the overall mean for any one characteristic were also culled. From the remaining families, the 20 best individuals were selected according to the following critieria:

a. A score of 4 or 5 for both the crook rating and overall rating.

b. Maximum height growth.

c. When two or more trees had approximately the same height, then the tree with the higher number of branches per whorl or darker needle color was favored.

d. No more than one tree from each family was selected.

The difference between the means of the 20 select trees and the overall mean for each characteristic was computed, resulting in the selection differential. Expected genetic gains were then computed and are presented in Table 3. For comparison the maximum expected gains were also computed for each characteristic. Between 84 and 96 percent of the maximum gain can be realized for the combined selection of crook, rating and height. Needle color should show some improvement with a very modest increase in the number of branches per whorl.

Table 3. Expected gains for selection for each characteristic individually and in combinatin with other characteristics.

Variable	Maximum gain	Select trees
Height	16.03 cm	13.51 cm
Crook	0.25	0.24
Color	0.56	0.21
Branches/whorl	0.20	0.06
Rating	0.33	0.28

The expected gain for most characteristics, such as height, has a definite clear meaning. The progeny from the select trees should exhibit an increase in height of 13.51 cm at age three. However, the expected gain in "quality rating" of 0.28 does not have a clear meaning. One method for obtaining a better understanding of the meaning and impact of this gain is

to study the rating frequencies which produced the observed mean and the changes which are anticipated to realize the expected gain.

The actual rating frequencies (adjusted to a per 1000 tree basis) are presented in Table 4. This frequency distribution produced the observed mean of 3.02 for the quality rating. If the expected gain is realized, then the progeny will have a mean of 3.30. A straight percentage increase of 29 percent from each rating to the next is required to achieve the new mean. This would result in the expected frequencies presented in Table 4.

Table 4. Actual and expected rating frequencies per 1000 trees.

Rating	Actual	Expected
1	93	66
2	191	162
3	374	321
4	288	313
5	54	138
Average Rating	3.02	3.30

Assuming that a 29 percent average increase from one rating class to the next is realistic, then there would be a 29 percent reduction in 1 rated trees which are all culls. There would also be approximately a 15 percent reduction in 2 rated trees - those which require major corrective procedures. It is impossible to correct all trees rated 2 to make them salable. In fact, it would be more accurate to assume that only 50 percent of 2 rated trees can be made into a salable product. A 29 percent reduction in 1 rated trees and a 15 percent reduction in 2 rated trees, assuming half of these are not salable, represents an average increase of 42 merchantable trees per 1000 or approximately per acre.

A clearer understanding and more meaningful measure of the above gains can be obtained by assigning a dollar value to them. Assuming a value of $2.00 per foot, or $12.00 per tree as a wholesale price for a 6-foot Virginia pine Christmas tree, an additional 42 trees marketed per acre represents an increase in income of $504.00. An increase in height of 13.5 cm on 853 trees per acre would represent a gain of $756.00. Of possible greater importance, but not easily measured, is the percentage of the trees that will be of better quality and will have required less maintenance. Also, more trees will be merchantable at an earlier age, increasing the rate of return on a grower's investment.

CONCLUSIONS

Although there were significant differences among seed sources of Virginia pine ranging from South Carolina to Mississippi and north to Tennessee, the differences were not large and accounted for less than 2.5 percent of the total variation observed in any of the characteristics which were evaluated. Seed source differences within this test area are not considered to be important. Based on the calculated estimates of heritabilities and potential gains, the genetic improvement of Virginia pine for Christmas trees is possible and practical. The greatest improvement can be achieved if the selection of superior trees primarily focuses on the characteristics of overall quality rating and stem straightness, followed by height growth with the least emphasis being placed on branches per whorl and needle color. Progeny from select trees should average 13.5 cm taller and result in approximately 4 percent fewer cull trees per acre, representing an increase in commercial value of over $1,200.00 per acre.

The Virginia pine is a species which is characterized by a large amount of tree-to-tree variation, which is particularly pronounced in juvenile trees. One objective of future research efforts should be to minimize the environmental source of variation within a test location. This variation causes reduced estimates of heritabilities and may mask much of any realized genetic gains without accurate measurements. The estimates of heritabilities for growth related factors may also be greatly influenced by site conditions, primarily soil conditions.

LITERATURE CITED

Brown, G. F. 1987. Estimates of genetic gain andselection stategy for Virginia pine Christmas tree characteristics. Ph.D. Dissertation, Dept. of Forestry, Wildlife, and Fisheries, The U. of Tenn., Knoxville, Tn. 99p.

Davis, T. S., J. W. Demski, and B. R. Murray. 1981. Critical factors in the Production of quality Virginia pine Christmas trees. Limbs and Needles. 9(3), 25-27.

Hu, S. and A. C. Main. 1980. Producting and marketing locally grown Christmas trees. Limbs and Needles. 8(2), 7-8.

Murray, B. R. 1983. Marketing outlook for the Christmas tree industry. In Christmas tree production and marketing. L.S.U. 32nd Annual Forestry Symposium, 114-121.

SAS Institute Inc. 1982. SAS User's Guide: Statistics. Cary, NC: SAS Institute Inc. 584p.

Warlick, C. O., S. E. Duba, and J. F. Goggans. 1985. Seed source variation in growth and ornamental traits of Virginia pine. Alabama Agr. Expt. Sta. Bull. 566, 23P.

A TREE IMPROVEMENT PROGRAM TO DEVELOP CLONES OF LOBLOLLY PINE FOR REFORESTATION

G.S. Foster[1] and D.V. Shaw[2]

Abstract.--The program emphasizes resistance to fusiform rust (Cronartium quercuum) (Berk.) Miyabe ex Shirai F. sp. fusiforme) as well as volume production of loblolly pine (Pinus taeda L). Parent trees are selected from a tested first generation population and crossed. A controlled-environment screening technique serves as an early selection for disease-free individuals and provides family information for later selection. Rust-free seedlings enter a vegetative propagation system using rooted cuttings. Clonal progeny tests, planted at multiple sites, provide data for later selection. Clonal selections are made using information derived from controlled-environment rust testing as well as field tests. The selections produce two populations: a clonal production population and a breeding population. The two populations may contain different subsets of selections since the one is selected mainly for general combining ability while the other is selected for total genetic value.

Clonally derived production plantings have become an intergal part of tree improvement programs where species are amenable to vegetative propagation. As a vehicle for reforestation, populations of clonal selections express genetic gains that are unattainable by traditional seedling propagation methods. Clonal selection and vegetative propagation utilize total genetic variation; therefore clonal programs can be opportunistic in capturing non-additive genetic variation as well as carefully planned to utilize additive genetic variation comparable to traditional programs. Examples of the results of selection and clonal propagation for reforestation include Norway spruce (Picea abies Karst.) (Roulund 1981), radiata pine (Pinus radiata D. Don) (Fielding 1970), and eastern cottonwood (Populus deltoides Bartr.) (McKnight 1970).

Techniques that are currently utilized by International Forest Seed Company enable large scale production of rooted cuttings of loblolly pine (Pinus taeda L.). Such vegetative propagation technology is being applied in combination with a recurrent selection program to develop clonal selections superior for both fusiform rust resistance and production traits.

---

[1] International Forest Seed Company, Odenville, Alabama 35120

[2] Assistant Professor, Department of Pomology, The University of California, Davis, CA 95616.

BASE POPULATION AND MATING DESIGN

One hundred and twenty-seven parent trees are selected from a pool of tested, first-generation selections from a combination of two sources: North Carolina State University-Industry Cooperative Tree Improvement Program, and the Cooperative Program between the U.S.D.A. Forest Service and the Georgia Forestry Commission. Traits for parental selection include superior resistance to fusiform rust and superior height growth as evidenced in progeny tests. The select trees are then mated at random using small disconnected factorials (generally 4 x 4).

EARLY SCREENING FOR RUST RESISTANCE

Seedlings from the resultant seed are then screened in the U.S.D.A. Forest Service Bent Creek Resistance Screening Center using standard techniques (Anderson et al. 1983). Data include percent of seedlings from each cross with a rust gall as well as other traits which are combined into a resistance score (C.H. Walkinshaw; U.S.D.A. For. Ser.; Principle Plant Pathologist; personal communication; gall traits defined by Walkinshaw et al. 1980). Seedlings which emerge from the screening with no rust symptoms then become the population for field testing.

The rust free survivors are planted in a cutting orchard at an approximate age of six months from seed. Henceforth, the trees are hedged at a height of about 1.5 ft. The hedging serves two purposes: 1. to maintain juvenility (Libby et al. 1972) and 2. to increase the number of potential cuttings (Foster et al. 1981).

FIELD TESTING

The field tests are designed to achieve a compromise between the total number of trees that can be planted and efficiencies of genetic value estimation for families and clones in families (Shaw and Hood 1983). Each tested clone is planted at three locations with two ramets per clone at each location, providing a total of six ramets per clone (3 locations x 2 ramets per location). At each location one ramet per clone is placed in each of two complete blocks with clones from a single family distributed among six blocks per location. This design achieves balance at the family level across replications and locations but is only partially balanced at the clone within family level. The testing program contains a maximum of 25 clones per family. The model utilizes factorials as sets of families (Hallauer and Miranda 1980). Clones from single families are distributed randomly within blocks and treated statistically as a single, non-contiguous family plot (Lambeth et al. 1980). Three check lots (N.C. State Cooperative) are included in each block as both seedlings and rooted cuttings.

In order to further screen the greenhouse-test survivors for fusiform rust resistance, each test is located in a high rust hazard area (over 50 percent infection) (Anderson 1986). Additionally the tests are geographically separate; such a distribution is intended to maximize sampling of divergent rust strains and promote selection for generally resistant clones.

Tests are measured annually for the first five years and again at 10 years. Traits measured include survival, presence of rust galls, height, d.b.h., and volume.

SELECTION AND PRODUCTION OF PROPAGULES

Initial selection of clones is based on the early screening data for rust incidence at the family level and third year field data for both rust incidence and height. Clones are selected for two populations (Foster 1986): 1. a breeding population and 2. a production population.

The initial breeding population contains approximately 300 clones selected for breeding value, using an index of multiple traits and a combination of family and individual information. This relatively early schedule for initial selection allows the clones to enter an accelerated breeding program (Greenwood 1983), while later culling can remove inferior clones. A further reduction of the select population (100 clones eliminated at age four and an additional 40 at age 5) depends on later measurements. Therefore the actual breeding of select trees entails only 160 selections (approximately the size of the initial population). The early selection procedure described expends effort in maintaining some clones that are never included in subsequent generations but shortens by two years the time required before crossing can be completed.

Two hundred selections are chosen for the production population based on third-year test data. These clonal selections depend only on genotypic value, rather than breeding value, derived from a multiple trait index. Hence simple, mass genotypic selection is employed (Foster 1986). Gain from genotypic selection utilizes total genetic variance, and therefore the selections for the breeding population may contain a subset of trees that overlaps only partially with the production population.

As with the breeding population, early selection in the production population provides necessary advanced warning to allow multiplication of the chosen clones with additional hedged ramets prior to final selection. A process of progressive elimination based on age 4 and 5 data is used to reach a final population of 50 clones. Early selection results in expended effort for increasing clones that eventually will be discarded. However, such an investment saves two years between selection and commercial scale propagation for the 50 clones eventually selected. Rapid deployment of advanced planting stock for reforestation is a very real advantage of clonal propagation (Matheson and Lindgren 1985).

ADVANCING THE BREEDING POPULATION

The same mating design as in the first generation is used for the second generation selections, with the exception of the matching of mates in which a a positive assortative mating arrangement is used (Foster 1986). The ranking of selections is based on their breeding values, and then the list is

subdivided into progressively descending groups of eight selections. These eight selections then constitute a 4 x 4 factorial, and selections are designated as male or female parents in alternating fashion down the list. This arrangement of mates helps to maximize the correlation of breeding values which is an important consideration in positive assortative mating (Crow and Felsenstein 1968). If related selections occur within a factorial, they are all assigned as the same sex to avoid potential crossing.

As with the mating design, the testing and selection schemes are duplicated from the previous generation.

CONCLUSIONS

The described program provides a system to greatly increase genetic gain in fusiform rust resistance and height growth in loblolly pine by using a recurrent selection scheme for the breeding population while using clonal propagation for the production population. Separation of the two populations and allocation of effort to different goals of selection result in steady, yet somewhat slower, gains from improving additive genetic effects in the breeding population; while the production population delivers maximum gain from the breeding population each generation. Selection of preliminary populations for both breeding and production purposes at age three, with subsequent culling based on later results, increases the flow of advanced cycle material into reforestation programs and shortens the generation interval.

LITERATURE CITED

Anderson, R.L, C.H. Young, and J.D. Triplett. 1983. Resistance screening center procedures manual: a step-by-step guide used in the operational screening of Southern pines for resistance to fusiform rust. (Rev. June 1983). USDA For. Serv., State and Private For., Report #83-1-18.

Anderson, R.L., N.D. Cost, J.P. McClure, and G. Ryan. 1986. Predicting severity of fusiform rust in young loblolly and slash pine stands in Florida, Georgia, and the Carolinas. South. J. Appl. For. 10(1):38-41.

Crow, J.F., and J. Felsenstein. 1968. The effect of assortative mating on the genetic composition of a population. Eugenics Qtrly. 15:85-97.

Fielding, J.M. 1970. Trees grown from cuttings compared with trees grown from seed (Pinus radiata D. Don). Silvae Genet. 19:54-63.

Foster, G.S. 1986. Making clonal forestry pay: breeding and selection for extreme genotypes. In: Proc. IUFRO Conf.; Joint Mtg. Working Parties on Breeding Theory, Progeny Testing, and Seed Orchards; Williamsburg, VA; Oct. 13-17, 1986. Pub. by N.C. State Univ. - Industry Coop. Tree Imp. Pgm. pp. 582-590.

Foster, G.S., F.E. Bridgwater, and S.E. McKeand. 1981. Mass vegetative propagation of loblolly pine - a reevaluation of direction. Proc. 16th South. For. Tree Imp. Conf. pp. 311-319.

Greenwood, M.S. 1983. Maximizing genetic gain in loblolly pine by application of accelerated breeding methods and new concepts in orchard design. Proc. 16th South. For. Tree Imp. Conf. pp. 290-296.

Hallauer, A.R., and J.B. Miranda. 1981. Quantitative genetics in maize breeding. Iowa State Univ. Press. Ames.

Lambeth, C.C., W.T. Gladstone, and R.W. Stonecypher. 1983. Statistical efficiency of row and noncontiguous family plots in genetic tests of loblolly pine. Silvae Genet. 32:24-28.

Libby, W.J., A.G. Brown, and J.M. Fielding. 1972. Effects of hedging radiata pine on production, rooting, and early growth of cuttings. New Zealand J. For. Sci. 2:263-283.

Matheson, A.C., and D. Lindgren. 1985. Gains from the clonal and the clonal seed-orchard options compared for tree breeding programs. Theor. Appl. Genet. 71:242-249.

McKnight, J.S. 1970. Planting cottonwood cuttings for timber production in the South. USDA For. Serv. Res. Pap. SO-60. 17 p.

Roulund, H. 1981. Problems of clonal forestry in spruce and their influence on breeding strategy. Commonwealth For. Bureau, Forestry Abstracts 42(10):457-471.

Shaw, D.V., and J.V. Hood. 1985. Maximizing gain per effort by using clonal replicates in genetic tests. Theor. Appl. Genet. 71:392-399.

Walkinshaw, C.H., T.R. Dell, and S.D. Hubbard. 1980. Predicting field performance of slash pine families from inoculated greenhouse seedlings. USDA For. Serv. Res. Pap. SO-160. 6 p.

AREA POTENTIALLY AVAILABLE TO A TREE: A RESEARCH TOOL

W. R. Smith[1]

Abstract.--Accounting for competiton influences on individual tree growth has been a stumbling block for many forest scientists. Partitioning the forest floor into areas potentially available (APA) to individual trees (Brown 1965) provides a useful competition index that has been overlooked or avoided. Two reasons for this are a lack of understanding of this geometric construct and its properties and inefficient methods of computation. Advances in computational geometry and forestry have made APA and similar geometric constructs practical and useful research tools in the assessment of competition. The creation and properties of these geometric models are discussed.

Additional keywords: polygon, triangulation, competition index.

INTRODUCTION AND BACKGROUND

Analysis of individual tree data is extremely difficult due to the confounding of competition with other influences. Numerous competition indices exist for both the average competition in the stand and the individual tree. The performance of many of these is limited because spatial information is not incorporated in the index. The area potentially available (APA) uses the spatial information of a tree's competitors to develop an index to account for the effects of local density on a tree's growth.

One of the earliest writings on the concept of APA was by the German forester Koenig in 1864. Though this concept has been in existence for over a century, only small advances in the application and utility of APA have been made. One reason for this is that many forest scientists are unacquainted with APA as a research tool. For those acquainted with APA, the reasons it is not used are its intractability and lack of flexibility for different applications.

The background and mathematical origins and properties of APA are presented herein. Advances in computational geometry, computer science, and forestry are used to develop APA so it can be easily used by forest scientists.

The shapes most frequently used in the past to define APA have been circles and squares (Opie 1968; Gerrard 1969; Bella 1971; Smaltschinski 1981). Brown (1965) was the first to use a polygon to represent APA and introduced the term. Jack (1967) independently developed APA for use in individual tree sampling. Fraser and Van Den Driessche (1972) were the first in forestry to express the mathematical origins of APA

[1] Research Forester, USDA Forest Service, Southern Forest Experiment Station, Institute for Quantitative Studies, New Orleans, Louisiana.

and used the "least diagonal neighbor" network for the estimation of stand density and spatial distribution. Fraser (1977) went on to adapt this tessellation, partitioning of the plane, for use in a method of unequal probability individual tree sampling that could be easily implemented in the field. Moore et. al., (1973) compared weighted APA to Bella's competitive influence-zone overlap (CIO) competition index. APA performed as well if not better than CIO. Moore also noted that APA when weighted will result in areas that are not assigned to a tree. Pelz (1978) used APA to predict individual tree growth. Johnson and Pelz (1979) investigated the use of APA and weighted APA for forest inventory. Daniels (1981) compared APA and weighted APA to other competition indices for the prediction of Basal area growth. Weighted APA performed the best when other significant variables were added for the prediction of basal area growth.

TRIANGULATIONS

APA is based upon a network of triangles. The primary function of the triangulation used for APA is to define competitors and form a geometric construct from which area can be assigned to an individual. A tree is a competitor if it is close to the subject tree, but not blocked from competing by a closer tree. One way of approximating this is to create a triangulation in which the sum of the length of the edges is the minimum. This triangulation is the minimum weight triangulation (MWT). No algorithm will consistently create the MWT. The two best heuristics for the MWT are the Delaunay triangulation (DT) and Greedy triangulation (GT) (Lloyd 1977; Gilbert 1976; Manacher and Zobrist 1979).

The basis of Brown's APA is the DT (figure 1). The definition of a DT is that all triangles form a circumcircle that contain no other points in the plane. The triangle network used by Fraser was the least diagonal neighbor network, better known as the GT. The GT is generated by creating all possible edges of a set of points. Then all edges that do not intersect a shorter edge are in the tessellation. One important aspect of both triangulations is that they are unique.[2] How well these triangulations approximate the MWT varies. The sum of the length of the edges of the DT can be $\Omega(N/\log N)$ as large as the MWT and the GT can be $\theta(N^{1/3})$ times greater than the MWT (Manacher and Zobrist 1979).[3]

---

[2] The GT will not be unique in the rare event two or more equilength edges intersect.

[3] This notation was developed by D. E. Knuth (1976) for the analysis of algorithms. The notation 'O(f(N))' means of the same or lesser order of f(N). 'θ(f(N))' means in exactly the same order as f(N) and 'Ω(f(N))' means in the same or greater order of f(N). This notation is generally used in reference to the speed and memory of an algorithm, though it may refer to other quantities. When referring to speed f(N) is the number of primary operations an algorithm must perform based on the number, N, of datum input. This notation with respect to memory indicates the amount needed.

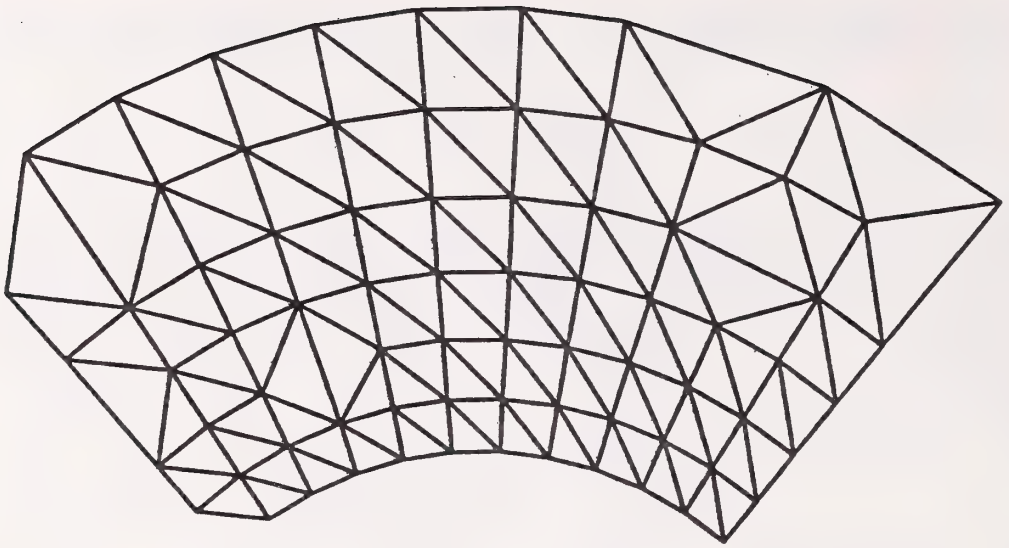


Figure 1. Delaunay triangulation for a portion of a Nelder's wheel. The vertices of the triangulation are the tree locations. The edges define a trees competitors.

Computational efficiency is another important criterion in the choice of a triangulation to create APA. Researchers in the fields of computational geometry and computer science have developed efficient algorithms and programs to create DT's and GT's. The GT can be generated in $O(N^2 \log N)$ run time in the worst case requiring $O(N^2)$ memory (Gilbert 1976). The DT can be created in O(NlogN) run time consistently and only requires O(N) memory (Lee 1980). The benchmark for evaluating the efficiency of these algorithms is O(NlogN), the optimal time for creating any triangulation (Shamos and Hoey 1975). The GT is a better heuristic to the MWT than the DT but is not as efficient for the calculation of APA.

The triangulation networks mentioned are models of first-order neighbors (FON), which are those trees identified as competitors by these triangulations due to their angle and euclidean distance relative to adjoining trees. The FON model assumes that distance and ability to compete are equivalent. This assumption is reasonable for plantations with fairly uniform growth. Heterogeneity in crown class and size could invalidate the use of FON as a model for the identification of competitors, ie., distance from a subject tree may not relate to the choice of the correct competitors. In situations in which the assumption of FON may be weak or incorrect, the triangle network may be used to identify an extended list of possible competitors, including second-order neighbors (SON). A second-order neighbor is the first-order neighbor of a first-order neighbor of a subject tree. Figure 1 is used to illustrate. Number the spokes from 1 to 12 (left to right), number the rows 1 to 7 (top to bottom). The notation (2:1) refers to the top tree on the second spoke from the left. Trees on spoke 7 and rows 3 and 4 are denoted (7:3,4). Tree (7:4) is the subject tree. Its FON's are trees (6:3,4), (7:3,5), and (8:4,5). Its SON's are trees (5:2,3,4), (6:2,5), (7:2,6), (8:3,6), and (9:4,5,6). The recursive nature of this definition makes the identification of any-order neighbor possible by use of the triangle network. The list of FON and SON should be adequate to identify the true competitors.

POLYGONS

APA as defined by Brown is the Voronoi polygon (VP). The VP can be created by calculating the intersections of the perpendicular bisectors of the edges in the DT. The VP was first applied by Thiessen and Alter (1911) for obtaining weighted estimates of rainfall from weather stations in a region. The primary property of the VP which made it useful to Thiessen and for modeling APA is that any point falling within the polygon is closer to the point for which the polygon was created than any other point, a reasonable heuristic for the zone of influence. Another aesthetically pleasing property of the VP is that all polygons have a convex hull, as do most tree crowns (figure 2).

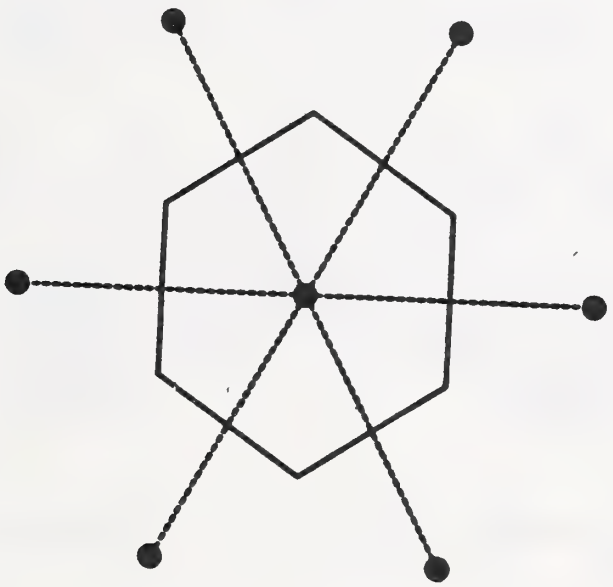


Figure 2. A Voronoi polygon for a diagonal spacing of 10 by 6.

In the same manner that the geometric model must be able to identify which trees can influence others, it must also be able to represent how strongly one tree influences another. The VP assumes that all trees, regardless of size, are equal in their ability to influence another tree. To avoid this dubious assumption, the model should be able to reflect the varying amount of influence exerted by different size trees. Weighting can be used to represent the influence one tree has on another. When these polygons are weighted, "gaps" and "overlaps" will appear between polygons. The idea of areas within a stand not being utilized is intuitively appealing; whether these unassigned and overassigned areas created by unconstrained weighting have any biological relevance has not been determined.

The sampling method developed by Fraser (1977) used a novel method of creating the vertices of the polygons. This method guarantees that there are no gaps or overlaps no matter which weights are used. The weighted midpoints of the edges

extending from a subject tree are calculated. The weighted midpoints are always on the hull of the polygon. A vertex between each adjoining edge of a subject is created by calculating the intersection of a line segment drawn from the weighted midpoint of the first edge to the neighbor defined by the adjoining edge and the line segment drawn from the weighted midpoint of the adjoining tree to the neighbor identified by the first edge.[4] In contrast to the VP, Fraser's polygons (FP) do not always contain the area closest to the subject, even when the weights are equal, and are not convex (figure 3). The properties of these polygons will be the same, no matter which triangulation is used as a basis.

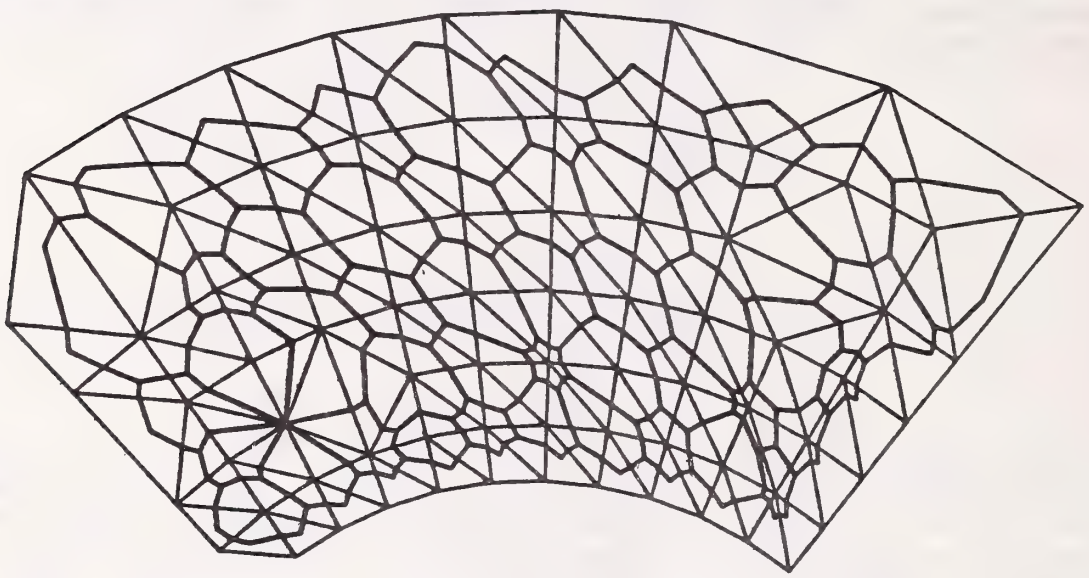


Figure 3. The same Delaunay triangulation as in figure 1 overlayed by Fraser polygons weighted by dbh^2.

Computing the VP and FP is a very simple and efficient task. Programs developed by Green and Sibson (1977) and Lee (1980) create the VP. The DT is, in fact, most efficently computed by first creating the VP. The Algorithm by Gilbert (1976) can be used to create the GT, though much less efficiently than the DT, and only a few simple procedures need be added to obtain the FP from either triangulation.

FLAWS AND A SOLUTION

There are several flaws and difficulties in creating APA using these geometric models. One difficulty is encountered by the use of FON and SON. The list is not the list of competitors for each subject tree but rather a list of candidates. Competitors must be selected by some criteria from this list. Secondly, the weighted

[4] See Fraser (1977) for further clarification.

or unweighted FP and unweighted VP will tessellate the entire stand without leaving gaps. The resulting APA can be excessive given the size of the tree. The weighted VP has the reverse flaw of causing biologically unjustified gaps and overlaps when weighted. One final difficulty is in creating the APA for border trees on the convex hull of the triangulation. To generate APA with the methods described requires that there not be a region of 180 degrees surrounding the subject tree void of competitors, the definition of a border tree.

To solve these problems, a theoretical limit of influence can be used. The limit defines which FON and SON candidates are capable of interacting by the intersection of their limits. The limit will also correct for gaps, overlaps, and excessive APA and will allow for the creation of APA for border trees. The selection of a limit should have strong biological justification due to its effect on the size and shape of the APA of a tree. The author has used the relationship of crown radius to dbh for open grown trees as a theoretical limit with biological justification. This relationship is species dependent (figure 4).

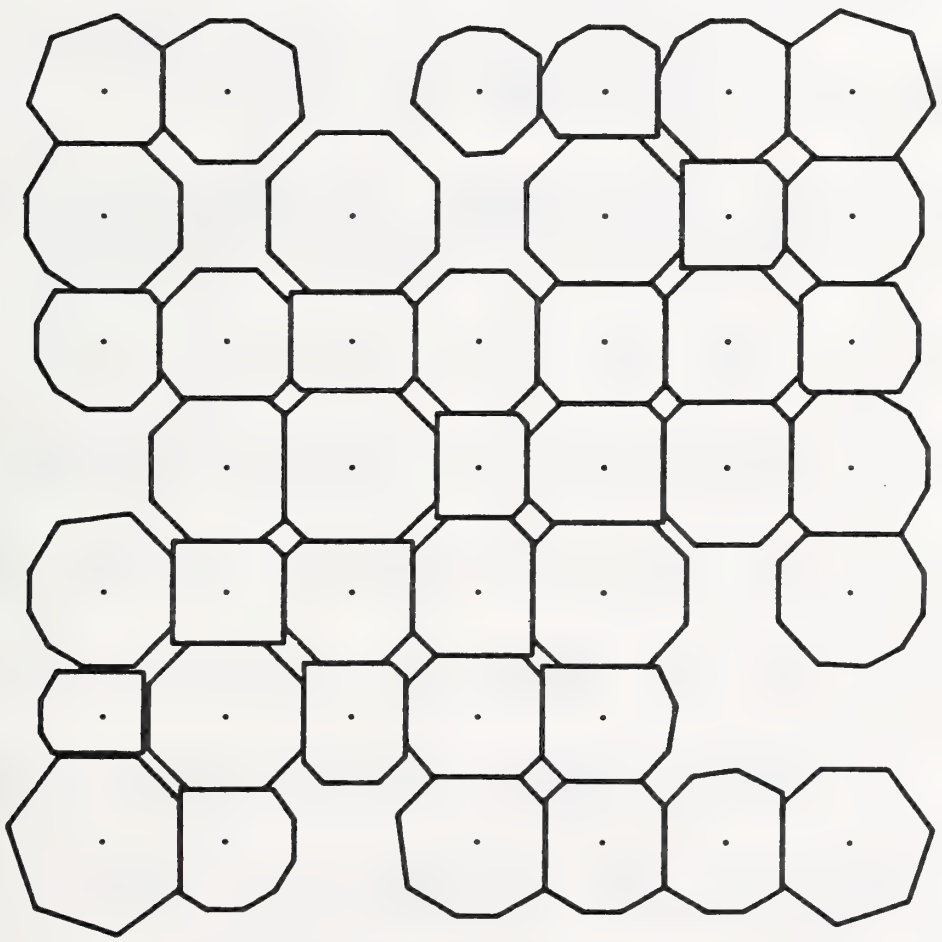


Figure 4. Weighted Voronoi polygons with crown radius versus dbh constraint for a spacing of 6 by 6 for a plantation of loblolly pine. The weight is dbh^2.

SUMMARY

Several methods of creating APA's and their properties were introduced. The methods used to create these polygons are demonstrated to be computationally efficient and flexible. The APA generated for a tree is shown to have many possible forms based on the assumptions of the spatial relationships of the trees in a stand.

LITERATURE CITED

Bella, I.E. 1971. A new competition model for individual trees. For. Sci. 17:364-372.

Brown, G.S. 1965. Point density in stems per acre. Forest Res. Inst., N.Z. Forest Service, For. Res. Notes No. 38, 11 p.

Daniels, R.F. 1981. An integrated system of stand models for loblolly pine. Ph.D. dissertation. Virginia Polytechnic. Institute and State University, Blacksburg, VA.

Fraser, A.R. and P. Van Den Driessche. 1972. Triangles, density and pattern in point populations. In: Proc. 3rd Conf. Advisory Group of Forest Statisticians, Int. Union For. Res. Organ., Inst, Nat. Rech. Agric., Jouy-en-Josas, France. p. 227-286.

Fraser, A.R. 1977. Triangle based probability polygons for forest sampling. For. Sci. 23:111-121.

Gerrard, D.J. 1969. Competition quotient: a new measure of the competition affecting individual forest trees. Mich. State Univ. Agric. Exp. Stn., Res. Bull. No. 20, 32 p.

Gilbert, P.D. 1976. New results on planar triangulations. M.Sc. Thesis. University of Illinois, Urbana, IL.

Green, P.J. and R. Sibson. 1977. Computing dirichlet tessellations in the plane. The Comput. J. p 168-173.

Jack, W.H. 1967. Single tree sampling in even-aged plantations for survey and experimentation. In: Proc. XIV IUFRO-Congress, Munich, Germany, p. 379-403.

Johnson, A.J. and D.R. Pelz. 1979. Tree polygons in forest sampling. University of Illinois at Urbana-Champaign Agric. Exp. Stn., For. Res. Rep. No. 79-5. 4 p.

Knuth, D.E. 1976. Big omicron and big omega and big theta. SIGACT News. 8(2):18-24.

Lee, D.T. 1980. Two dimensional voronoi diagram in the Lp-metric. J. ACM. pp. 604-618.

Lloyd, E.L. 1977. On triangulations of a set of points in the plane. M.Sc. Thesis. Massachusetts Institute of Technology, Cambridge, MA.

Manacher, G.K. and A.L. Zobrist. 1979. Neither the greedy nor the delaunay triangulation of a planar point set approximates the optimal triangulation. Inf. Process. Lett. 9(1):31-34.

Moore, J.A., C.A. Budelsky, and R.C. Schlesinger. 1973. A new index representing individual tree competitive status. Can. J. For. Res. 3:495-500.

Opie, J.E. 1968. Predictability of individual growth using various definitions of competing basal area. For. Sci. 14:314-323.

Pelz, D.R. 1978. Estimating individual tree growth with tree polygons. In: Growth Models for Long Term Forecasting of Timber Yields (Ed. J. Fries, H.E. Burkhart, T.A. Max). School of Forestry and Wildlife Resources, Va. Polytech. Inst. and State Univ. FWS-1-78. p. 172-178.

Shamos, M.I. and D. Hoey. 1975. Closest point problems. in: Proc. 16th IEEE Annu. Symposium on the Foundations of Computer Science. p. 208-215.

Smaltschinski, T. 1981. Density and distribution structure of forest stands. Ph.D. dissertation. Albert-Ludwig University. Freiburg, Germany.

Thiessen, A.J. and J.C. Alter. 1911. Precipitation averages for large areas. Monthly Weather Review. 39:1082-1084.

DYNAMICS OF STAND GROWTH AND YIELD OVER 29 YEARS IN A LOBLOLLY PINE SOURCE TRIAL IN ARKANSAS

C. B. TALBERT and M. R. STRUB[1]

Abstract. Stands representing 36 unselected, rangewide sources of loblolly pine in a 29-year-old geographic source trial in southwestern Arkansas were compared in terms of stem diameter, dominant height, mortality and stand basal area production trends over time. Significant geographic-source differences were maintained through age 29 for Dbh and dominant height, with eastern sources (particularly East Coast) maintaining an approximately constant percent superiority of 3-8% after age 11. By the study's conclusion the superiority of eastern-origin trees over western in volume per tree was 18%. Late mortality differed little between East Coast and local Arkansas stands through age 26, but subsequently diverged. Stand basal area production was superior in East Coast origin stands through age 26, but declined to no significant difference by age 29. The observed declines in yield superiority of East Coast over local stands after age 26 could not be shown to result from any difference in "competitive ability"; rather, it is hypothesized that the faster-growing East Coast stands reached a similar maximum size-density trajectory four years sooner. Given the value and cost advantages of larger piece size, these results favor use of East Coast material on moist sites in Arkansas. However, for the early growth benefits of source movement to be maintained through the long term, the fast-growing eastern-origin stands must be aggressively managed to minimize yield reductions associated with severe competitive stress.

INTRODUCTION

A very few organizations have begun to deploy and manage select Atlantic-coastal loblolly pine families on substantial plantation acreages in Arkansas and Oklahoma, on the basis of long-term seed source trials which have demonstrated a considerable superiority of Atlantic-coast material over local in individual-tree yield and form without major added mortality (Wells and Lambeth, 1979; Lambeth et al., 1984).

Strategic planning and forest management decisionmaking with respect to these non-local stands depend on an accurate assessment of expected stand-yield performance and response to competition through time. Stand yield data for trials near commercial rotation ages remains scarce. A scheduled thinning of a 29-year-old (from seed) loblolly pine source trial in southwestern Arkansas in early 1985 provided an opportunity to compare stand development for local and non-local stands grown together.

MATERIALS AND METHODS

The source trial discussed here was planted in 1957 in southwestern Arkansas in a cooperative effort between the Southern Forest Experiment Station, Potlatch Corporation and Georgia-Pacific Corporation. The portion of the study used in this analysis consists of 36 rangewide sources of loblolly pine, each planted in one 49-tree-square plot (with two borders) in each of four non-adjacent blocks in Hempstead County, Arkansas (Figure 1). These sites could be considered moist for Arkansas, with moisture-holding capacities of 20-23 cm. A "light thinning from below" (no other information available) was performed just before the 17th growing season from seed. Individual-tree yield, survival, fusiform rust infection and stem form results were reported through field-age 10 by Grigsby (1973), for field-age 16 by Grigsby (1977), and for field-age 25 by Wells and Lambeth (1983).

---

[1] Weyerhaeuser Company, Biological Sciences Research Division, Tacoma WA and Southern Forestry Research Division, Hot Springs AR

In early 1985, five trees were chosen from each source-plot to approximately represent the range of Dbh for that plot. Total height, Dbh, taper and wood specific gravity were measured on each stem. Total height, Dbh and taper were used in Smalian's formula to compute a 'true' total volume per tree. In addition, the two largest-Dbh stems per plot were sectioned longitudinally, and annual heights measured. Data collection was a cooperative effort, involving representatives from Potlatch Corporation, Weyerhaeuser Company, the U.S. Forest Service, Oklahoma State University and Texas A&M University. The 1985 data were then combined with historical data from ages 11, 17 and 26 (graciously provided by O. Wells and C. Lambeth) for this analysis.

The 36 sources were further delineated into seven geographic "regions" (Figure 1) for analysis, following the outlines used by Wells and Lambeth (1983). Analyses of variance were performed using the GLM procedure of SAS to assess the magnitude and pattern of region differences over time in individual tree and stand yield attributes. In addition, region differences in "competitive ability" were evaluated through a statistical test of heterogeneity in level and slope of region size-density trajectories between ages 26 and 29. Specific comparisons among regions and region groupings of interest were performed using single d.f. contrasts within the analyses of variance.

RESULTS AND DISCUSSION

Individual-Tree Yield

Region rankings and the significance of region differences for Dbh remained quite consistent from age 11 through age 29 (Table 1, Figure 2). Trees from regions east of the Mississippi River, and from the East Coast region in particular, were significantly larger in diameter than local and other western origin trees throughout this trial. Gulf Coast and Interior source trees did not differ significantly from East Coast source trees in Dbh after age 11, but trees from the Northeast showed a trend toward increasing slenderness relative to other eastern sources as the study progressed.

Region differences in dominant height[1], particularly between eastern and western sources and between East Coast and local sources, also were statistically significant at all four ages examined in this analysis (Table 1, Figure 3). The percentage difference between East Coast and local sources was greatest in the early years of the study (through age 17), although the absolute difference increased through age 26 and was essentially constant thereafter. This result supports the observation of Sprinz et al. (1987a), from the same study, that region height-age curves differed in shape, with the eastern sources growing more rapidly early on, but the western sources showing a "growth spurt" later, leading to essentially parallel height-age curves by the end of the study. The four "eastern" sources (Northeast, East Coast, Gulf Coast and Interior) did not differ significantly from one another in dominant height at any of the ages examined.

The observed region rankings for Dbh and height were reflected in similar rankings for "true" volume per tree at age 29 (Table 1). Again, eastern sources averaged much larger than western sources (0.33 m^3 vs. 0.28 m^3), and East Coast origin trees averaged larger than local-origin trees (0.32 m^3 vs. 0.27 m^3). Northeast origin trees ranked with the top sources for volume per tree, despite their small average diameter, not only because of their height but also because of excellent stem form - trees from the Northeast were significantly more cylindrical than trees from any other region (Sprinz et al., 1987b).

---

[1] Dominant height is defined here as the average height of the two largest-diameter trees on a 49-tree plot.

FIGURE 1. Location of geographic sources and boundaries of geographic regions used in the analysis (after Lambeth et al.).

Stand Yield

Mortality behavior for the different regions after age 11 tended to follow an inverse trend with average stem diameter, with some notable exceptions (Table 2). Between ages 17 and 26, Gulf Coast and Interior stands (two of the largest in Dbh during that period) suffered considerably more mortality than most of the other regions. However, the Lost Pines source, which ranked near the bottom for Dbh at ages 17 and 26, showed the second-greatest percent mortality during that period, while East Coast origin stands, which ranked near the top for Dbh, exhibited very little mortality during that period (no significant difference from local stands). During the period from ages 26 to 29, Gulf Coast, Interior and Lost Pine stands continued to exhibit relatively high mortality percentages, and East Coast stands also began to show added mortality relative to local stands. Stands of Northeast origin, despite an improving rank for Dbh from age 26 to 29, actually exhibited less mortality than local Northwest origin stands between those ages.

Table 3 and Figure 4 combine individual-tree yield and mortality performance for the seven regions in terms of stand basal area and volume yield. Two trends are most apparent in these data. The first is the poor stand yield performance of Gulf Coast and Interior stands after the age-17 thinning, a reflection of the high mortality observed in stands from these regions. The second is the changing stand-yield superiority of East Coast origin material during the study period. The statistically significant 8.5% stand basal area superiority of East Coast stands over local stands observed at age 17 declined to 6.6% (ns) by age 26 and -1% (ns) by age 29. Total volume per hectare was slightly greater for East Coast stands than for local stands (1.6%, ns) at age 29, due to the height and stem form superiority of East Coast material at that age.

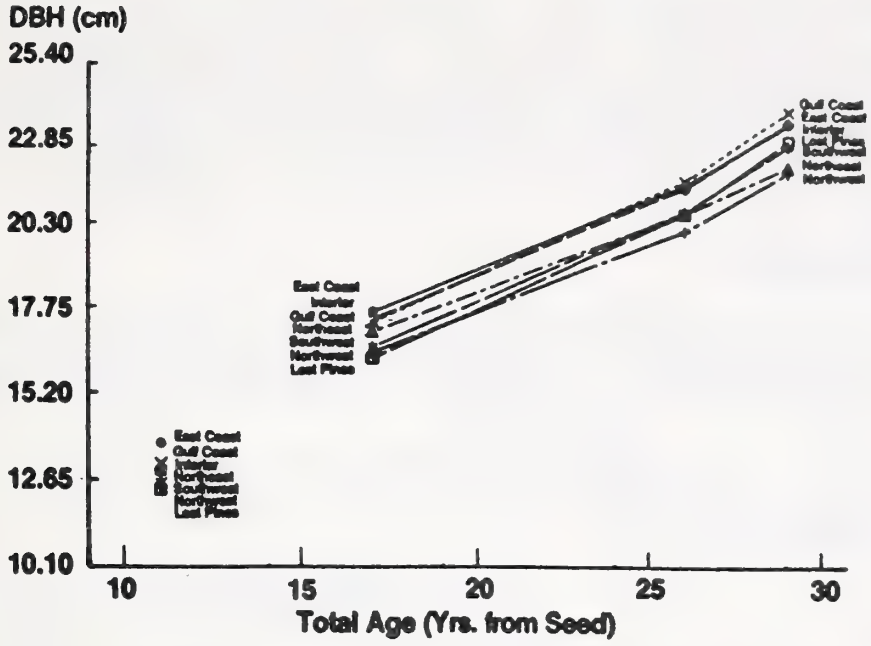


FIGURE 2. Time-trend in plot-average Dbh for 7 geographic regions of loblolly pine in an Arkansas provenance trial.

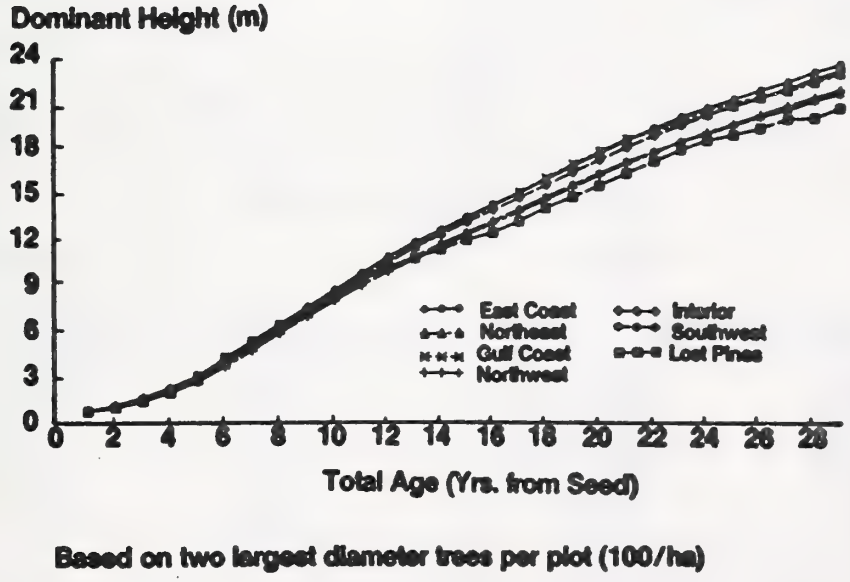


FIGURE 3. Time-trend in dominant height for 7 geographic regions of loblolly pine in an Arkansas provenance trial.

Source variability within a region was large and statistically significant for percent mortality and for stand basal area during the entire study period. In fact, source-in-region variability was actually greater than region-to-region variability for these traits. This result indicates that, even within a poor-performing region, sources (families?) can be found which perform well, and conversely, that care should be taken to test a number of sources, even from a high-ranking region, to screen out poor sources prior to large-scale deployment.

Response to Competition

Why a decline in the stand production advantage of East Coast stock after age 26? Is this a reflection of some kind of late-onset adaptability problem which is causing greater-than-expected mortality or less-than-expected growth in East Coast stands grown in Arkansas?

TABLE 2. Percent mortality[a] for seven geographic regions of loblolly pine in an Arkansas provenance trial.

Source	DF	TIME INTERVAL (Age from Seed) 17 to 26 P>F	26 to 29 P>F
BLOCK	3	***	****
REGION	6	***	ns
East vs. West		ns	ns
East Coast vs. Northwest		ns	ns
Gulf Coast vs. East Coast		**	ns
Interior vs. coastal		ns	ns
Northeast vs. East Coast		ns	ns
SOURCE (reg)	29	*	**
ERROR	103	4.21[c]	2.48

	TIME INTERVAL (Age from Seed) 17 to 26 $\bar{x}$		26 to 29 $\bar{x}$
Region	(%)	Region	(%)
GC	23.6	GC	14.2
LOST	18.9	LOST	12.4
INT	16.0	EC	12.2
SW	10.3	INT	11.2
NE	8.4	SW	11.2
EC	8.41	NW	6.6
NW	7.1	NE	5.4

[a] Percentage variable was transformed to arsine (sqrt (%)) for analysis of variance.

[b] ****: Significant at $\alpha = 0.0001$
***: Significant at $\alpha = 0.01$
**: Significant at $\alpha = 0.05$
*: Significant at $\alpha = 0.10$
ns: Not Significant

[c] Sum of squares is listed for ERROR

TABLE 3. Stand basal area and volume yield for seven geographic regions of loblolly pine at ages 11, 26 and 29 from seed, in an Arkansas provenance trial.

	Age from Seed						
	11		26		29		
Source	DF	Basal Area (m^2/ha) PR>F[a]	DF	Basal Area (m^2/ha) PR>F	DF	Basal Area (m^2/ha) PR>F	Volume/Tree (m^3/ha) PR>F
BLOCK	3	****	3	****	3	****	***
REGION	6	ns	6	***	6	**	**
East vs West	1	ns	1	ns	1	ns	ns
EC vs NW	1	ns	1	ns	1	ns	ns
GC vs EC	1	ns	1	***	1	***	***
INT vs Coast	1	ns	1	ns	1	ns	ns
NE vs EC	1	ns	1	ns	1	ns	ns
SOURCE (REG)	29	**	29	**	29	***	ns
ERROR	103	122.9[b]	103	4199.0	103	5681.5	1238084.8

	Basal Area (m^2/ha)		Basal Area (m^2/ha)		Basal Area (m^2/ha)		Volume (m^3/ha)	
	Region	$\bar{x}$	Region	$\bar{x}$	Region	$\bar{x}$	Region	$\bar{x}$
	EC	30.3	EC	44.3	NW	46.1	NE	394.2
	INT	29.4	NE	42.0	EC	45.7	EC	337.5
	NE	29.2	SW	41.6	NE	45.5	NW	332.2
	SW	28.9	NW	41.6	SW	45.5	INT	326.2
	LOST	28.9	INT	40.2	INT	42.2	SW	300.3
	NW	28.7	LOST	37.0	LOST	40.0	GC	275.8
	GC	27.6	GC	34.7	GC	35.8	LOST	254.8

[a] ****: Significant at $\alpha = 0.001$ **: Significant at $\alpha = 0.05$ ns: Not significant
***: Significant at $\alpha = 0.01$ *: Significant at $\alpha = 0.10$

[b] Sum of squares is listed for ERROR

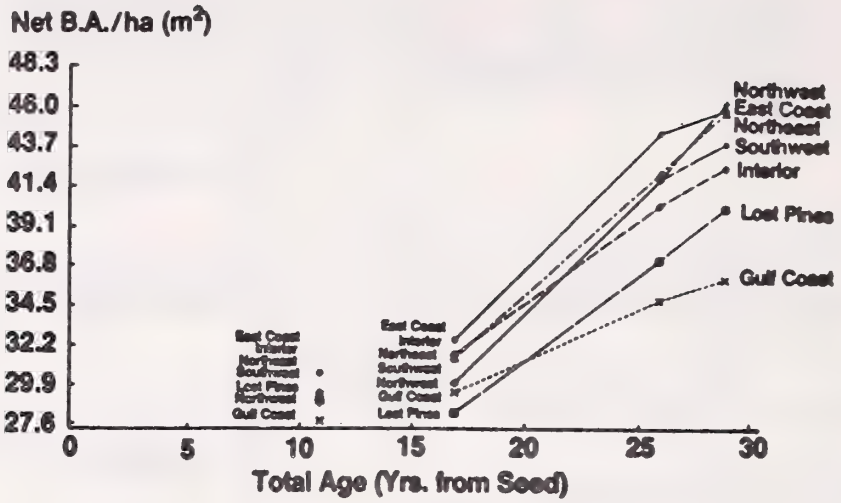


FIGURE 4. Time-trend in stand basal area per hectare for seven geographic regions of loblolly pine in an Arkansas provenance trial.

The basis for evaluating these possibilities lies in the fundamental concept of maximum size-density. The relationship of average plant volume or weight with stocking in log-log space is remarkably consistent - a linear trend with a slope of -3/2 - across a wide variety of species, from weeds to forest trees (Yoda et al., 1963; Kira et al., 1953; Drew and Flewelling, 1979; Reineke, 1933).[2] Once a given stand has reached full site occupancy, it progresses through time by moving along a size-density "trajectory," with increases in stem size (growth) accompanied by decreases in stand density (mortality). Although the slope of the size-density relationship appears to be consistent across species, the level of the relationship varies widely.

The slope and level of the size-density relationship for local source stock could be viewed as a benchmark for the amount of mortality which "adapted" material would experience per unit increase in average tree size, and for the maximum average tree size which could be expected in an "adapted" stand at a given stand density. Non-local stands exhibiting a flatter slope would be showing more mortality than local stands per unit of size growth. Non-local stands showing a lower level would be demonstrating an inability to carry the same average size as local stands were their stocking levels the same.

In this study, when only age-26 and age-29 data were included, all region stands with two exceptions followed size-density trajectories which were consistent with Reineke's (1933) -1.605 slope. Local and Northeast stands exhibited size-density slopes which were significantly steeper (less mortality per unit of size-growth) than the others (Figure 5). It is a distinct possibility, however, that the size-density slopes for the local and Northeast stands would have "flattened" to near -1.605 had the study continued past age 29. Certainly the amount of competitive stress (as indicated by stand basal area) being experienced by stands from these regions at age 26 was considerably less than the amount being experienced by East Coast stands at the same age. It is not possible to

[2] When Dbh is used as the dependent variable rather than volume, Reineke (1933) showed that a -1.605 slope applied, across several tree species.

draw a definitive conclusion on size-density slope differences from these data. However, it is clear than Gulf Coast and Interior stands exhibit size-density trends which are considerably lower in level than the size-density trends exhibited by the other regions in the study. Stands of these two regions appear unable to maintain the same average tree size for a given stocking level that stands of the other regions carried.

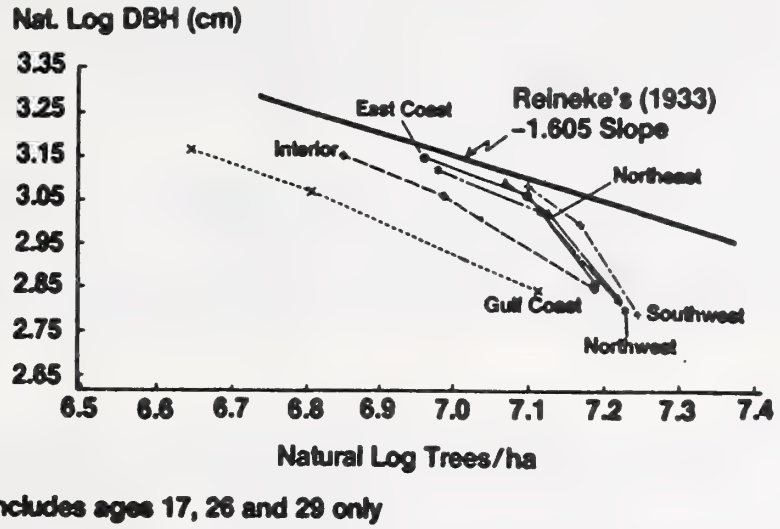


FIGURE 5. Size-density trajectories for six geographic regions (Lost Pines not included) of loblolly pine in an Arkansas provenance trial.

CONCLUSIONS

The use of East Coast material on moist sites like these in Arkansas appears to offer substantial benefits over use of local material, both in individual-tree and stand yield, through stand densities which could be considered quite high for loblolly pine. There does, however, appear to be a point in stand development at which severe competitive stress results in a decline in the stand-yield superiority of East Coast material (after age 26 or 44.3 m^2/ha. of stand basal area in this study), and the earlier yield advantages thereafter are reduced.

It cannot be demonstrated conclusively from these results that East Coast stands exhibit an intrinsically lower "carrying capacity" or exhibit more mortality per unit of growth than local-origin stands grown on the same sites. Instead, it is very likely that the decline in the stand-yield superiority of East Coast stands after age 26 only reflects a "normal" response to the much higher levels of growing stock in the East Coast stands relative to local origin stands of the same age. Of course, this conjecture should be restricted to the types of moist sites represented by the study blocks - drier sites could well lead to very different relative responses.

The strong relationship of piece-size to net stand value should also be considered here. As the average Dbh of a stand increases, harvesting and handling costs per unit of volume tend to decrease sharply, while product value per unit volume tends to increase with average diameter for solidwood uses. Therefore, there would appear to be a stand-value benefit to use of East Coast stock over the smaller diameter local stock even for the same stand-volume yield.

The Gulf Coast sources used in this trial exhibited excessive mortality when grown this far offsite, even though the study sites were moist. Any decision to use Gulf Coast stock in Arkansas should be carefully considered in light of this evidence. Also, Interior sources of loblolly pine do not appear to offer significant long-term advantages in survival or growth rate over East Coast sources for planting on moist sites in Arkansas on the basis of this study. Diameter growth consistently averaged less, and mortality the same or greater, throughout the study period. On the other hand, Northeast-origin stands actually outyielded both local and East Coast origin stands in total volume by the conclusion of this study, and were superior in stem form. Further testing of stands from this region is warranted.

The results of this study underscore the critical interdependence of forest management and genetic improvement. For the early yield advantages of source movement to be realized, faster-growing non-local stands clearly must be thinned or harvested before they reach severe levels of competitive stress, and such treatments may be required sooner in the faster-growing stands than in slower-growing local stands.

LITERATURE CITED

DREW, T. J. and J. W. FLEWELLING. 1979. Stand density management: an alternative approach and its application to Douglas-fir plantations. For. Sci. 23:518-32.

GRIGSBY, H. C. 1973. South Carolina best of 36 loblolly pine seed sources for southern Arkansas. USDA For. Serv. Res. Pap. 50-89, 10pp.

GRIGSBY, H. C. 1977. A 16-year provenance test of loblolly pine in southern Arkansas. In: Proc. 14th S. For. Tree Impr. Conf., Gainesville, Fla.:261-268.

KIRA, T., H. OGAWA and N. SAZAKI. 1953. Intraspecific competition among higher plants. I. Competition-yield-density inter-relationship in regularly dispersed populations. J. Inst. Polytechnics, Osaka City Univ., Series D. 4:1-16.

LAMBETH, C. C., P. M. DOUGHERTY, W. T. GLADSTONE, R. B. MCCULLOUGH and O. O. WELLS. 1984. Large-scale planting of North Carolina loblolly pine in Arkansas and Oklahoma: A case of gain vs. risk. J. For., Dec. 1984:736-741.

REINEKE, L. H. 1933. Perfecting a size-density index for even-aged forests. J. Agr. Res. 46:627-638.

SPRINZ, P. T., C. B. TALBERT and M. R. STRUB. 1987a. Height-age trends from an Arkansas seed source study. Manuscript submitted to Forest Science.

_____. 1987b. Relationships among tree dimensions from an Arkansas seed source study. In preparation.

WELLS, O. O. and C. C. LAMBETH. 1984. Loblolly pine provenance test in southern Arkansas: 25th year results. S.J. Applied For. 7(2):71-75.

YODA, K., T. KIRA, H. OGAWA and K. HOZUMI. 1963. Self-thinning in overcrowded stands under cultivated and natural conditions. J. Biol., Osaka City Univ. 14:107-129.

POLYMIX CROSSES FOR RUST RESISTANCE SCREENING

by T. D. Byram[1/], W. J. Lowe[2/], and C. R. McKinley[3/]

Abstract--Six slash pine families with different levels of resistance to fusiform rust were crossed with two polymix pollens and tested at the Resistance Screening Center in Asheville, N.C. The polymixes consisted of pollen from disease resistant or disease susceptible clones. Resistance Screening Center scores from the resistant polymix crosses did not agree with family rankings in moderately to heavily infected field tests ($r = -0.22$). However, the susceptible polymix crosses grouped parents in approximately the same order as field trials ($r = -0.85$).

This research supports the assertion that a polymix composed of inferior parents will more accurately separate females if the trait of interest is controlled by dominant genes. Pollen from rust susceptible rather than resistant clones should be used to produce crosses with slash pine parents that are to be tested at the Resistance Screening Center.

Keywords: Slash pine, Pinus elliottii Engelm. var. elliottii, Cronartium quercuum f. sp. fusiforme

INTRODUCTION

Fusiform rust (Cronartium quercuum f. sp. fusiforme) is the most damaging disease on slash pine (Pinus elliottii Engelm. var. elliottii) in the Western Gulf region of the United States. In 1980, 55 percent of the slash pine trees in Southeast Texas were infected (Pase 1980). This was a dramatic increase from 1976 when just over 40 percent of the slash pine in the same counties were diseased (Walterscheidt and Van Arsdale 1976). Similar increases in the occurrence of fusiform rust have been reported across the south (Griggs and Schmidt 1977). Because infected trees often die (Nance et al. 1981) or have decreased product value, increased resistance to fusiform rust is the most important trait for slash pine breeding in the Western Gulf Forest Tree Improvement Program (WGFTIP).

1/Assistant Geneticist, Western Gulf Forest Tree Improvement Program, Texas Forest Service, College Station, Texas.

2/Associate Geneticist, Texas Forest Service and Assistant Professor, Texas Agricultural Experiment Station, College Station, Texas.

3/Associate Geneticist, Texas Forest Service and Assistant Professor, Forest Science Dept., Texas A&M University, College Station, Texas.

To improve resistance while maintaining selection pressure for growth and form characteristics, the WGFTIP has implemented a two step testing program. All slash pine selections are initially evaluated for disease resistance at the Resistance Screening Center in Asheville, North Carolina, (Anderson and Powers 1985) and only the resistant parents are utilized in the traditional breeding program.

In the past, all seed submitted from the Western Gulf Forest Tree Improvement Program to the Resistance Screening Center was collected from either the ortet or a seed orchard. Under these circumstances, it was assumed that the pollen source was random and that females would be correctly ranked. A moderate correlation coefficient ($r = -0.73$) between Resistance Screening Center scores and field data (Byram et al. 1982) indicated that this assumption was adequate. Most selections currently being tested are located in scion banks where a random pollen source may not be present. This makes it necessary to use either single-parent control-pollinations or polymix crosses to produce seed for testing. Because the only goal is to rank parents, polymix crosses have been chosen.

The objective of this study was to determine if the males selected for the polymix would affect the ability to rank females for resistance to fusiform rust at the Resistance Screening Center.

MATERIALS AND METHODS

Twenty-six slash pine clones were selected on the basis of known resistance to fusiform rust. The percent of trees infected with either a limb or stem gall was determined for each family. This percent was converted to standard deviations compared to the average fusiform rust infection in the plantation and averaged across progeny tests. To be included in the clonal evaluation, a minimum of 30 percent rust infection and differences among families at the 10 percent level of significance were required for each test. Families with negative scores had less rust and were considered to be more resistant. Six clones were chosen as female parents. The remaining 20 clones were used as male parents and divided into rust resistant and susceptible polymix groups. Field trial scores and the number of observations included are listed in Table 1.

Polymix pollen was formulated by mixing equal weights of clean dry pollen from each male parent. All six females were crossed with both polymix groups and the resulting 12 seedlots were submitted to the Resistance Screening Center (RSC). Positive RSC scores indicated rust resistance at the Resistance Screening Center. RSC scores for the resistant and susceptible polymix crosses were independently regressed against the field scores.

Table 1. The average field score for fusiform rust expressed as standard deviations (SD) from the test mean and the number of tests in each observation for all parents included in the study.

Females			Resistant Polymix			Susceptible Polymix		
Name	SD	No. of Tests	Name	SD	No. of Tests	Name	SD	No. of Tests
S5PC8	-0.926	2	K-143	-0.726	3	K-141	1.389	4
K-211	-0.492	6	BSS-13	-1.100	5	K-142	1.042	5
D12PC10	-1.226	4	BSS-10	-1.351	4	K-163	0.906	2
S5PC3	0.607	7	C-103	-1.717	1	OI-S-1	0.614	4
S5PC6	0.809	4	OI-S-4	-1.024	6	OI-S-5	0.803	2
D12PC29	0.987	3	OI-S-2	-0.722	3	S6PC1	1.710	2
			S5PC5	-1.319	9	D4PC71	2.050	2
			D5PC44	--	--	D5PC57	1.590	2
			D5PC98	-2.065	2	D5PC228	0.486	2
			D5PC286	-1.419	3	S2PC1	0.212	7

RESULTS AND DISCUSSION

There was no significant relationship between RSC scores for the resistant polymix crosses and field data; however, the correlation between the RSC score for the susceptible polymix crosses and field data was significant ($r = -0.85$) (Figure 1). The RSC scores for the susceptible polymix crosses correctly separated the resistant and susceptible female parents and ranked them the same as the field tests with only one exception.

Polymix crosses should adequately rank parents for general combining ability. However, the type of gene action controlling the trait of interest will determine which parents should be used for the polymix. If disease resistance is determined by dominant genes, then a resistant polymix would mask differences among females by contributing dominant genes (resistance) to the progeny. In this case, a susceptible polymix should be used because the female's dominant genes for resistance would not be masked by the pollen contribution. If resistance is determined by recessive genes, then a resistant polymix would provide a better separation among females than a susceptible polymix. This data indicated that resistance to fusiform rust in slash pine was controlled by dominant genes and the susceptible polymix was the correct choice.

When a small correlation between RSC and field scores has occurred, it may be partially due to the level of rust resistance in the pollen. Females crossed with resistant pollen or by pollens with varying degrees of resistance may be incorrectly ranked. This incorrect ranking could occur at the Resistance Screening Center, and/or in field tests. Controlling the contribution of the male parent by using

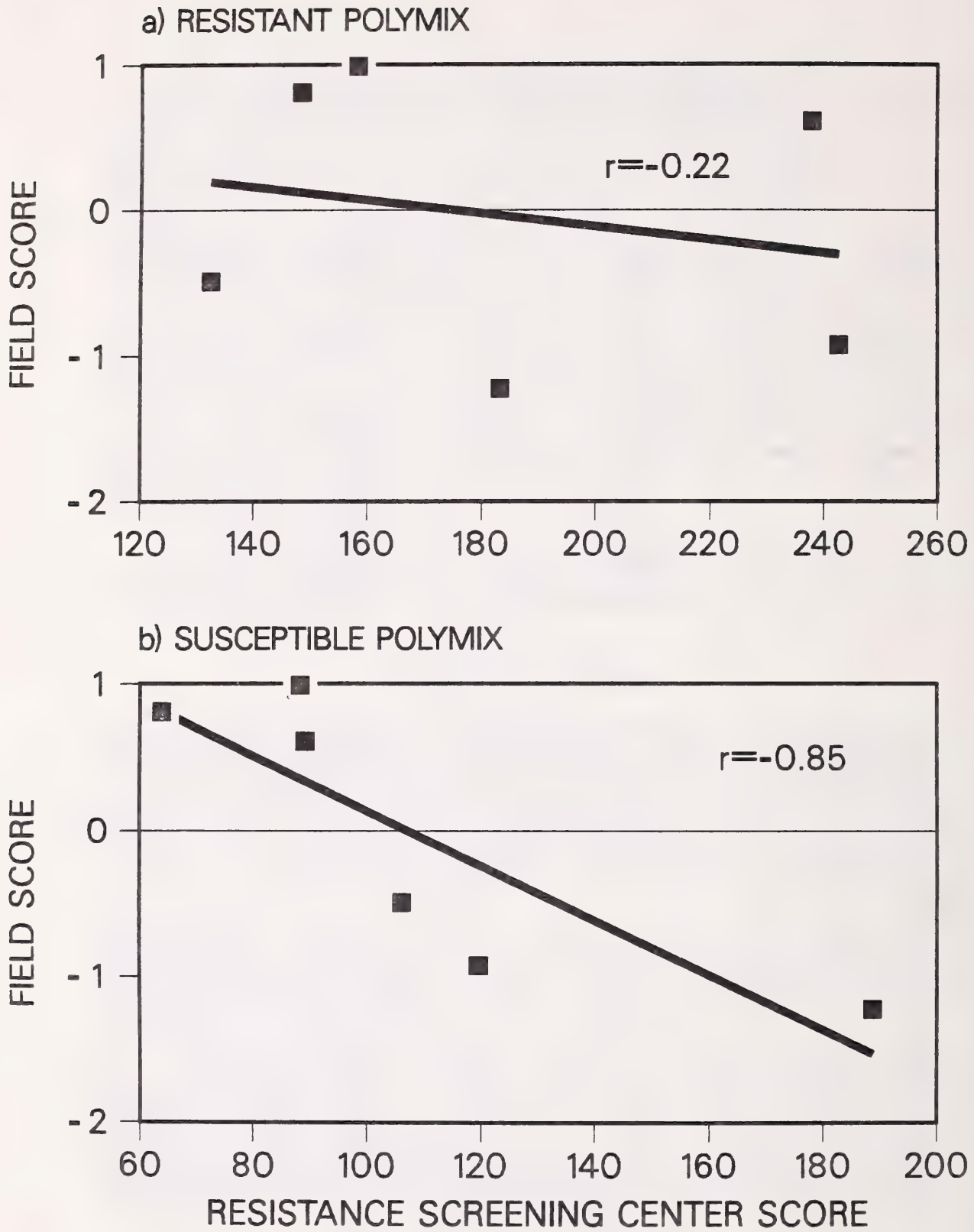


Figure 1. Relationship between rust resistance in the field (standard deviations to the plantation average) and Resistance Screening Center scores for the same six females crossed with a) resistant polymix and b) susceptible polymix.

a standardized polymix also has the advantage of allowing more accurate clonal comparisons across organizations.

A polymix composed of inferior performing male parents should insure that dominant desirable traits in the progeny originated from the female parent. While this theory should apply to other traits and other species, an inferior polymix may not always be desirable in an operational tree improvement program. In cases where the traits of interest are related to seed set, viability or overall fitness, an average polymix may be more suitable. An example is the testing of second generation loblolly pine selections in the Western Gulf Forest Tree Improvement Program. Selections will be ranked for their general combining ability for volume growth based on crosses with a polymix pollen formulated from average parents. If a decision is made to use average or inferor parents for a polymix, the parents should be selected before they are rogued from the program.

CONCLUSION

A rust-susceptible polymix provided a better ranking of slash pine families at the Resistance Screening Center than a rust-resistant polymix. This indicated that resistance to fusiform rust in slash pine was controlled by dominant genes. The use of a constant susceptible polymix should provide a better ranking of slash pine families at the Resistance Screening Center and allow for more accurate comparisons among different organizations in the Western Gulf Forest Tree Improvement Program.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the personnel from Temple-Eastex Forests, the Texas Forest Service, Owens-Illinois and Kirby Forest Industries who made a significant contribution to this study by collecting pollen and performing polymix crosses.

Literature Cited

Anderson, R. L. and H. Powers, Jr. 1985. The resistance screening center - screening for disease resistance as a source for tree improvement programs. IUFRO Proc. The Rusts of Hard Pines Working Party Conference, Athens, GA. 1984. pp. 59-65.

Byram, T. D., W. J. Lowe, C. R. McKinley, J. F. Robinson, A. Stauder and J. P. van Buijtenen. 1982. 30th progress report of the cooperative forest tree improvement program. Texas Forest Service, Cir. 262. 25 p.

Griggs, M. M. and R. A. Schmidt. 1977. Increase and spread of fusiform rust. Symposium Proc., Mgt. of Fusiform Rust in Southern Pines. Univ. of Florida, Gainesville. pp. 32-38.

Nance, W. L., R. C. Froelich and E. Shoulders. 1981. Effects of fusiform rust on survival and structure of Mississippi and Louisiana slash pine plantations. USDA, For. Ser., Res. P. SO-172. 11 p.

Pase, H. A., III. 1980. Texas fusiform rust survey. An unpublished report by the Texas Forest Service. 15 p.

Walterscheidt, M. J. and E. P. Van Arsdale. 1976. Distribution of fusiform rust on slash and loblolly pines in Texas. Plant Disease Reporter 60:718-720.

REGENERATION OF YELLOW-POPLAR FROM PROTOPLAST CULTURE

S. A. Merkle and H. E. Sommer[1/]

Abstract.--Protoplasts were isolated from suspension cultures of two embryongenic lines of yellow-poplar (Liriodendron tulipifera) by incubation in a medium containing 2 percent cellulase and 1 percent pectinase, and cultured in droplets of a regeneration medium supplemented with 1 mg/l 2,4-D and 0.25 mg/l 6BA and solidified with agarose. The protoplasts reformed cell walls within 3 days, and 75 percent of them divided at least once within 1 week in culture. Embryogenic suspension or callus cultures were regenerated by placing agarose-solidifed droplets containing protoplast-derived microcalli directly into liquid conditioning medium or onto plates of conditioning medium solidified with agar. Embryogenesis was obtained by transferring the callus to a hormone-free induction medium, and protoplast-derived plantlets were obtained following transfer of somatic embryos to a germination medium. We believe this to be the first report of regeneration of a North American tree species from protoplast culture via somatic embryogenesis.

Additional keywords: Sómatic embryogenesis, tissue culture, Liriodendron tulipifera.

Since the first reported enzymatic isolation of plant protoplasts by Cocking (1960), protoplasts have been isolated and cultured from a wide range of higher plant species. With the appropriate techniques, cultures of plant protoplasts have achieved cell wall formation, cell division, colony and callus formation and plantlet regeneration. Protoplasts isolated from several herbaceous plant species have been fused to yield somatic hybrids (Evans et al. 1983). Protoplasts also open possibilities for the uptake of nuclei, organelles, chromosomes and plasmids (Ahuja 1984). Finally, protoplast cultures have been cited as a source of novel genetic variation, which might provide a method for plant improvement (Shepard et al. 1980).

Protoplasts have been isolated from several woody plant species including both gymnosperms and angiosperms (Ahuja 1984). Sources of protoplasts have included leaf mesophyll, cotyledonary tissue, callus cultures and suspension cultures. However, there are very few reports of the successful regeneration of whole plants from protoplast cultures of woody species, although the list has recently begun to expand more rapidly. Among those woody species successfully cultured from protoplasts are Citrus sp. (Vardi et al. 1982), Santalum album (Rao and Ozias-Akins 1985), Pyrus communis (Ochatt and Caso

[1/]Assistant Professor and Associate Professor, respectively, School of Forest Resources, University of Georgia, Athens, Georgia 30602.

1986), Ulmus sp. (Sticklen et al. 1985, 1986), Broussonetia kazinoki (Oka and Ohyama 1985) and Populus sp. (Russell and McCown 1986). In this paper, we will describe the isolation and culture of protoplasts of yellow-poplar and the regeneration of plantlets from these cultures via somatic embryogenesis.

MATERIALS AND METHODS

Aggregates of immature yellow-poplar samaras were collected at weekly intervals from July through September from 12 trees located on the University of Georgia campus and the University's Whitehall Forest. Samaras were dissected from the aggregates and surface sterilized using a 70 percent ethanol dip, followed by a 10 percent Roccal dip, a full strength Clorox soak for 5 minutes, a sterile water rinse, a 0.01 N HCl rinse and three additional sterile water rinses. Following surface sterilization, the embryo and endosperm were removed and placed on Merkle and Sommer's (1986) conditioning medium, supplemented with 2 mg/l 2,4-D, 0.25 mg/l 6BA and 1000 mg/l casein hydrolysate, and solidified with 0.8% agar. Cultures were incubated in the dark at 22°C and transferred every 3 weeks to fresh conditioning medium.

Within 2 months after being placed in culture, an embryogenic callus was initiated from a percentage of the cultures, which varied with both the mother tree and the date of seed collection. The embryogenic callus could easily be distinguished from nonembryogenic callus by its nodular, friable structure, fast growth and pale yellow color. Suspension cultures were established by subculturing this callus into liquid conditioning medium. Suspension cultures were maintained in 125 ml erlenmeyer flasks on a gyratory shaker at 90 rpm. As long as the callus or suspension was maintained on the 2,4-D-supplemented conditioning medium, no differentiation of somatic embryos occurred.

Prior to protoplast isolation, embryogenic suspension cultures were put on an accelerated transfer schedule, so that about 5 g of embryogenic material was transferred to fresh medium every 6 days. These accelerated subcultures were carried out at least 4 times prior to protoplast isolation, and resulted in very rapid growth of the suspension. Cultures to be used for protoplast isolation were divided into a dark pretreatment and a light pretreatment (14 hours of light per day).

To isolate protoplasts, approximately 1 g of suspension was placed in 10 ml of filter-sterilized digestion medium containing major salts, minor salts and iron as in Merkle and Sommer's (1986) conditioning medium, 500 mg/l $CaCl_2 \cdot 2H_2O$, 100 mg/l MES, 0.5 M mannitol, 2 percent Cellulysin, 1 percent Macerase and 100 mg/l bovine albumin. The tissue was incubated in the digestion medium for 12 hours at 30°C with gentle agitation on a gyratory shaker at 50 rpm. Following digestion, the protoplasts were purified by dripping the suspension, which contained protoplasts, undigested cells and debris, through 2 layers of Miracloth, then through a 25 µm pore size stainless steel sieve. The filtrate was centrifuged at 100 X g for 5 minutes. The supernatant was discarded and the pellet resuspended in 5 ml of filter-sterilized wash medium, which was of the same composition as the digestion medium, minus the enzymes and bovine albumin. This washing step of centrifugation and resuspension in wash medium was repeated twice more.

Following the final washing centrifugation, the supernatant was removed and the pellet of protoplasts was resuspended in a filter-sterilized protoplast culture medium, which was of the same composition as Merkle and Sommer's (1986) conditioning medium, but supplemented with 500 mg/l $CaCl_2 \cdot 2H_2O$, 250 mg/l xylose, 250 mg/l fructose and only 250 mg/l sucrose, instead of the 40 g/l used in the conditioning medium. The concentration of 2,4-D was also cut from 2 mg/l to 1 mg/l in the protoplast culture medium. The osmoticum for the medium was 0.5 M glucose. Since centrifugation resulted in clumping of the protoplasts, the resuspended protoplasts were dripped once more through a 25 µm sieve to disperse the protoplasts from the clumps. The protoplasts were counted using a hemacytometer and cell wall digestion was confirmed by Calcofluor staining.

Although other plating techniques were attempted, including thin liquid layer and hanging droplet methods, the high density plating technique of Binding and Kollman (1985) proved to be the most successful. Following this method, the protoplasts were diluted to 2-4 X 10^5 protoplasts per ml, pipetted into 0.2 ml drops in plastic petri dishes and mixed with an equal amount of protoplast culture medium containing 2.5% low melting point agarose, which had been autoclaved and cooled to 38°C. The mixture was immediately taken up with a pipette and redistributed as 0.05 ml droplets around the perimeter of the plate. Following solidification of the droplets, the plates were flooded with 2 ml of liquid protoplast culture medium. Thus, while the density of the protoplasts in the agarose droplets was 1-2 X 10^5 per ml, the actual plating density per dish was much lower.

Petri dishes containing the agarose droplet cultures were placed in inverted Magenta boxes to maintain humidity and incubated in darkness at 30°C for approximately 1 month. Then the culture medium was diluted by an equal volume of liquid yellow-poplar conditioning medium containing no osmoticum. One month following this dilution, agarose droplets containing calli were either transferred to 40 ml of liquid conditioning medium in 125 ml erlenmeyer flasks and maintained at 90 rpm on a gyratory shaker, or placed directly onto agar-solidifed conditioning medium in plastic petri dishes.

RESULTS AND DISCUSSION

Embryogenic suspension cultures grown under light conditions and transferred to fresh media every 6 days yielded 1-2 X 10^6 protoplasts per gram of tissue. Significant numbers of protoplasts could not be isolated from dark-grown suspension cultures. Protoplasts isolated from light-grown cultures (Figure 1) were very dense and filled with large starch granules. Because of their high density, protoplasts could not be separated from undigested cells by flotation, as can protoplasts derived from other tissues, such as leaf mesophyll. Because of this problem, purification was accomplished by the relatively long (12 hours) digestion time, during which virtually all free cells were converted to protoplasts, and by the sieving described earlier.

Once in culture, cell wall regeneration, as determined by Calcofluor staining and loss of spherical shape, occurred within three days. The first cell divisions were observed within 4 days and within one week of culture,

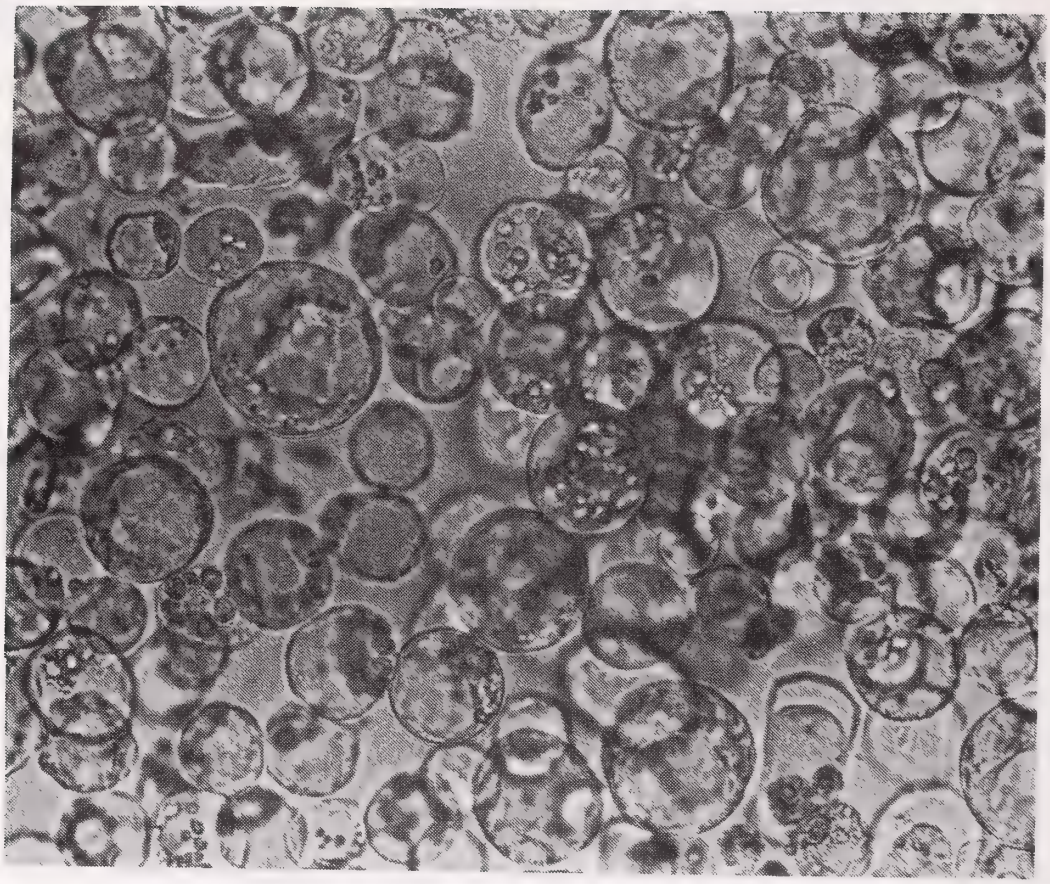


Figure 1. Protoplasts recently isolated from embryogenic yellow-poplar suspension culture (300X).

over 75 percent of the protoplast-derived cells had divided at least once. Many divisions were asymmetrical and budding was frequently observed. Cell colonies of 8 or more cells could be found within 2 weeks and microcalli large enough to be seen without a microscope appeared within 3 weeks. These regeneration times are quite rapid when compared to those reported for protoplasts isolated from shoot cultures of Populus, which took 6 weeks for the first cell division (Russell and McCown 1986) and protoplasts isolated from callus cultures of Ulmus x 'Pioneer,' which required 7 days (Sticklen et al. 1986). However, the regeneration timetable for yellow-poplar protoplast cultures was very similar to that reported for Santalum (Rao and Ozias-Akins 1985).

Suspension cultures could readily be regenerated from microcalli by transferring the agarose droplets containing the microcalli into 20 ml of liquid yellow-poplar conditioning medium in 50 ml flasks on a gyratory shaker. Within a few weeks, the microcalli expanded out of the agarose and proliferated rapidly in suspension. Agarose droplets containing microcalli were also placed directly onto conditioning medium solidified with agar, where

the microcalli grew out of the agarose and onto the conditioning medium. The callus and suspensions regenerated from protoplast cultures were identical in appearance to embryogenic cultures described by Merkle and Sommer (1986).

Embryogenesis was induced by transferring the callus to Merkle and Sommer's (1986) induction medium, which is of the same composition as the conditioning medium, except for the absence of hormones. Within 1 month following transfer of callus to the hormone-free medium, differentiation of somatic embryos could be observed.

Germination of the somatic embryos was accomplished as described previously (Merkle and Sommer 1986). Briefly, somatic embryos at the late torpedo stage were transferred to a Risser and White's (1964) medium in test tubes. Root growth was usually observed within one week, followed by development of 2-3 heart-shaped leaves. At this stage, plantlets could be transferred from the tubes to a peat-perlite potting mixture and placed in the humidifying chamber for acclimatization. During the following 6 weeks in the humidifying chamber with 14 hour daylengths and weekly fertilization with commercial plant food, the humidity was gradually lowered to ambient. Currently we have a few dozen plantlets of protoplast-somatic embryo origin growing in the greenhouse.

The availability of a system to regenerate yellow-poplar from protoplast culture via somatic embryogenesis opens many possibilities for basic and applied research using this tool. An obvious application of this system is the testing of techniques for the genetic engineering of forest species by Agrobacterium Ti plasmid-mediated DNA transformation or by direct DNA transfer enhanced by electroporation. The fact that the yellow-poplar system is an embryogenic one makes it an excellent candidate for studies into the commercial scale up of transformation-regeneration systems for forest trees, for example using bioreactor technology. An additional area for potential research using the products of protoplast research is the investigation of somaclonal variation among the regenerants. Although we have begun to look at within-clone variation at the DNA level among plantlets regenerated from yellow-poplar somatic embryos (Chou et al. 1986), a similar study of protoplast-derived plantlets will have to wait until a larger number of plantlets have been regenerated from the protoplast system.

LITERATURE CITED

Ahjua, M. R. 1984. Protoplast research in woody plants. Silvae Genet. 33:32-37.

Binding, H. and R. Kollman. 1985. Regeneration of protoplasts. In: In vitro techniques: propagation and long term storage (A. Schäfer-Menhur, ed.). Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht. pp. 39-99.

Chou, P. L., S. A. Merkle and H. E. Sommer. 1986. Repetitive sequences in the DNA of the genome of Liriodendron tulipifera tissue cultures. In: Proceedings of the 1986 TAPPI Research and Development Conference. TAPPI Press, Atlanta, GA. pp. 95-97.

Cocking, E. C. 1960. A method for the isolation of plant protoplasts and vacuoles. Nature 187:962-963.

Evans, D. A., J. E. Bravo and Y. Y. Gleba. 1983. Somatic hybridization: fusion methods, recovery of hybrids and genetic analysis. In: Plant protoplasts. Supplement 16 of the International Review of Cytology. (K. L. Giles, ed.) Academic Press, New York. pp. 143-159.

Merkle, S. A. and H. E. Sommer. 1986. Somatic embryogenesis in tissue cultures of Liriodendron tulipifera. Can. J. For. Res. 16:420-422.

Ochatt, S. J. and O. H. Caso. 1986. Shoot regeneration from leaf mesophyll protoplasts of wild pear (Pyrus communis var. Pyraster L.) J. Plant Physiol. 122:243-249.

Oka, S. and K. Ohyama. 1985. Plant regeneration from leaf mesophyll protoplasts of Broussonetia kazinoki Sieb. (paper mulberry). J. Plant Physiol. 119:455-460.

Rao, P. S. and P. Ozias-Akins. 1985. Plant regeneration through somatic embryogenesis in protoplast cultures of sandalwood (Santalum album L.). Protoplasma 124:80-86.

Russell, J. A. and B. H. McCown. 1986. Culture and regeneration of Populus leaf protoplasts isolated from nonseedling tissue. Plant Sci. 46:133-142.

Shepard, J. 1982. The regeneration of potato plants from leaf cell protoplasts. Sci. Amer. 246:154-166.

Sticklen, M. B., R. D. Lineberger and S. C. Domir. 1985. Isolation and culture of protoplasts of Ulmus x 'Homestead.' Plant Sci. 41:117-120.

Sticklen, M. B., S. C. Domir and R. D. Lineberger. 1986. Shoot regeneration from protoplasts of Ulmus x 'Pioneer.' Plant Sci. 47:29-34.

Vardi, A., P. Spiegel-Roy and E. Galun. 1982. Plant regeneration from Citrus protoplasts: variability in methodological requirements among cultivars and species. Theor. Appl. Genet. 62:171-176.

THE NATURE OF WATERLOGGING TOLERANCE OF LOBLOLLY PINE

D. D. Hook and T. Shear[1/]

Abstract.--Loblolly pine seedlings develop many responses under waterlogging stress that are similar to adaptations to waterlogging in other higher plants. Some of the responses of loblolly pine seem to be less specialized than those exhibited by true wetland species. Lack of specialization is some cases seems to account for growth differences in relation to soil nutrient level and length and type of waterlogging. Screening for various adaptations appears to have some potential for early progeny testing for wet site loblolly pine genotypes.

Additional keywords: gas exchange, iron-phosphorus complexes, morphology, anatomy and metabolism

Within its natural range loblolly pine is most prevalent on mesic sites but in the Atlantic and Gulf coastal plains individual trees frequently survive and grow well in shallow swamps, sloughs, wet flats, and other marginal wet sites. Consequently, many people including myself have theorized that wet site variants of loblolly pine exist throughout most of its range. However, scientific evidence to support this belief is lacking or, at most, is inconclusive.

Williams and Bridgewater (1981), in comparing loblolly pine families from deep peat and coastal plain sites found only a small family response to water regimes and that except for number of lateral roots, morphological traits and phytomass were statistically nonsignificant. Byram et al (1984) and Yeiser and Paschke (1987) reported that survival of some loblolly pine families was higher than others on wet sites. However, Yeiser and Paschke (1987) found no relationship between origin of ortet and family survival rates on wet sites. Topa and McLeod (1986a and b) found that a wet-site loblolly pine source from Robeson County, NC outgrew a dry-site source from Texas under waterlogging conditions and had a better internal ventilation system, but did not differ in phosphorus uptake rates. At least one loblolly pine seed orchard exists in the South for improving loblolly pine performance on wet sites (i. e., the wet-site loblolly pine seed orchard of Federal Paper Board Company, Lumberton, NC).

In summary, genetic variation appears to occur in loblolly pine populations with regard to waterlogging tolerance but whether the variation is large enough to be important in breeding and tree improvement programs has not been documented in the literature. Nor have there been sufficient comprehensive studies undertaken to determine the physiological and genetic basis of such variation.

1/ Professor and Director and Research Associate, respectively, Forested Wetlands Research Program. Clemson University and the Southeastern Forest Experiment Station, 2730 Savannah Highway, Charleston, SC. 29407.

The traditional approach of using genetic tests and a seed orchard is obviously being used by Federal Paper Board Company and perhaps others that I am not aware of. If we are to expand our horizons in improving loblolly pine performance on wet sites three questions need to be addressed. They are: 1) is there significant variation among families with regard to performance on wet sites; 2) can physiological responses be correlated to performance in such a manner that they can be used for screening tools in selecting for waterlogging genotypes; 3) can physiological studies provide information that will help guide future breeding and tree improvement programs?

No conclusive answers are available at this time but preliminary results on loblolly pine family performances and physiological responses to waterlogging stress have been encouraging (see Shear and Hook in these proceedings).

Before proceeding, it seems necessary to briefly review what is know about morphological and physiological adaptations of higher plants to waterlogging stress.

ADAPTATIONS OF PLANTS TO WATERLOGGING.

Seed and Growth Dormancy

Plants that normally grow on wet sites generally differ from more mesophytic plants in many ways. Depending on the species, seed may germinate under water (rice) or may remain inactive until the water recedes below the soil surface (baldcypress and tupelos). Germination under water may be beneficial or hazardous depending on depth or duration of inundation (Kramer and Kozlowski 1979). Rice seed germinate under water and send up a coleoptile that must extend above the water level to provide an avenue for oxygen to diffuse to the seed before the germination process can proceed. It gains a growth advantage over many of its competitors by this mechanism. Baldcypress and tupelos seed remain dormant until flood waters recede, germinate, and go through a rapid height growth phase for one or two years. Presumably this rapid height growth phase is advantageous in getting their foliage high enough to be above the next flooding event. Other species do not leaf out until late May or early June hence they partially avoid early season stress by this strategy.

Morphology and Anatomy

Species such as baldcypress and tupelos develop a number of morphological and anatomical adaptations under flooding stress. They develop butt swell (hypertrophy), knees, and water roots. These adaptations appear to be beneficial for rapid growth but may not be necessary for survival. On the other hand, anatomical traits that accompany morphological developments appear to be necessary for life in such environments. Lenticel proliferation on the submerged stem and roots and the concurrent development of intercellular spaces within the stem and roots appear to be a vital part of a ventilation system necessary for growth in waterlogged environments. The ventilation system is so well developed in baldcypress and the tupelos that oxygen readily diffuses from the atmosphere through the stem and roots into the surrounding soil. Consequently, their roots essentially live in a micro-aerobic

environment within soils that are devoid of oxygen and loaded with many reduced-toxic compounds.

Metabolism

Plants that live in waterlogged soils also have metabolic adaptations not found in their dry land counterparts. Variations in basic metabolism among species seem to be related to the degree of waterlogging stress that each is capable of tolerating. Most plants tested to-date have shown that ethanol fermentation is the primary pathway for anaerobic metabolism under oxygen stress. Despite considerable efforts to understand how and to what degree anaerobic metabolism and internal aeration play in plant adaptation to waterlogged environments, their integrated role is still unclear.

It is known, for instance, that oxygen diffusion from the atmosphere to the roots in well adapted wetland plants, such as Spartina alterniflora, is not sufficient to explain their growth performance on some sites. Furthermore, there is evidence that anaerobic metabolism via ethanol production helps maintain the energy level in their roots high enough to support most active metabolic processes (Mendelssohn, McKee, and Patrick, 1980). In contrast, it is known that no cell growth and only limited protein production occurs in the absence of oxygen (Webb and Armstrong, 1983 and Vartapetian, 1987). Thus, the ventilation and the anaerobic metabolism systems appear to be intricately linked within plants that are adapted to wetlands but how is not clearly understood. During the past seven years, myself and several colleagues have sought answers to: 1) how does loblolly pine adapt morphologically and physiologically to soil waterlogging and 2) what characteristics of the soil and water regime trigger adaptive responses? The results of these studies will be summarized below.

GENERAL TECHNIQUES

Short-and long-term pot studies in growth chambers and in glasshouses and long-term studies (from one growing season to three growing seasons duration) in the soil tanks and in the field have been used to assess the response of loblolly pine to continuous waterlogging, seasonal waterlogging, periodic waterlogging, well drained conditions and natural water regimes on wet sites with and without phosphorus (McKee and Wilhite, 1986; Hook et al, 1983; DeBell et al, 1984; McKee et al., 1984; McKevlin et al., in press; Hook and McKevlin, 1987; Shear and Hook, 1987; and Hook et a, in prep). In addition, Hook and Denslow (1987) evaluated the response of four loblolly pine populations to two water regimes with and without phosphorus for one growing season in the soil tanks.

The soil tanks are structures at the Santee Experimental Forest Headquarters near Huger, South Carolina designed specifically to study the influence of soil waterlogging on tree species. These facilities consist of six tanks with dimension of approximately 6'x 6'x 6'. They are constructed such that water levels and flow rates can be maintained at desired levels and rates within or above the soil. The soil within the tanks are usually added so that they tend to mimic the structure of natural soil profiles on wet sites (see Hook, Stubbs, Langdon, and Brown, 1970).

RESULTS AND DISCUSSION

Most plant species develop outward evidence of waterlogging stress (Hook 1984) and hardwood species usually develop signs of stress rapidly under waterlogged conditions (Hook and Brown 1971). In comparison to hardwoods, loblolly pine responds to waterlogging stress very slowly. Except for a slight yellowing of foliage, eventual loss of some foliage and a relative slow growth rate, it does not express obvious signs of adapting or dying during the first year. Because of its slow response to stress, it is not possible to detect differences in family performance during the first year of waterlogging stress. The slow response time prolongs early screening trials.

Survival

Loblolly pine has remarkably high survival under continuous soil saturation, particularly, if inherent soil phosphorus levels are high or if sufficient phosphorus is added to the soil. Several studies have shown that first year survival under waterlogging stress will generally be 90 % or higher whether the experiment is done in pots in growth chambers or glasshouses, in the soil tanks, or in the field on wet sites. Even after three years in the field, survival averaged 83% on moderately wet sites and 70% on very wet sites. McKee and Wilhite (1986) found at age 10 years, loblolly pine survival was 73% on a poorly drained site but with added phosphorus or bedding plus phosphorus survival was 87%. Hence, early survival seems to have limited value in evaluating the tolerance of loblolly pine to wet sites.

Growth

of loblolly pine is generally decreased by soil waterlogging but its performance under waterlogged conditions is closely tied to the level of available phosphorus in the soil. When soil phosphorus level is adequate, loblolly pine growth rate under severe waterlogging stress may be as good as in well drained conditions. McKee and Wilhite (1986) found that fertilization with potassium and phosphorus, initially and nitrogen at age 6 years, increased height growth by 95% and volume growth by three to four fold. Since potassium had no effect on growth and the addition of nitrogen did not change the growth curve, they attributed most of the response to phosphorus effects. Hook and Denslow (1986) found large differences in growth rate of loblolly pine with and without phosphorus under two water regimes during the first growing season but could not find a family response to waterlogging and there were no significant family x phosphorus interactions.

Morphology, Anatomy, and Internal Aeration

Waterlogging results in a slight hypertrophy of the stem near the water level and lenticels develop on the roots and lower stem of loblolly pine seedlings. Occasionally some seedlings develop adventitious roots but the most common occurrence is for roots to die back to the main root or secondary roots and develop roots that are morphologically and anatomically different from the original roots. Some of the newly formed roots develop large aerenchyma zones distal to the apical meristem of the root. The aerenchyma zones are connected to the lenticels on the stem via intercellular spaces in the pericycle of the root and the cortex of the stem (McKevlin et al in press).

Gas exchange occurs rapidly through the aeration system (i. e., gas exchange between the atmosphere and the roots can be detected within one minute of changing the atmosphere around the shoots). Furthermore, the aeration status of the root can change completely from anaerobic to aerobic or vice-versa within 15-to-30 minutes in response to controlled changes in the atmosphere around the shoot. Despite the apparent rapid rate of gas exchange between the shoot and root, the system is not efficient enough to oxidize the rhizosphere of loblolly pine roots as do water and swamp tupelos (Hook et al, 1971; Hook and McKevlin, 1987).

Metabolism and Nutrition

Soil waterlogging reduces the uptake of all mineral elements except iron. If phosphorus is added to the soil prior to waterlogging, nutrient uptake increases but the concentration of nutrients still remains below the concentrations found in seedlings growing in well drained soils.

The solubility of iron increases significantly in waterlogged soils. Most wetland species avoid excessive uptake by oxidizing a portion of the excess iron on the outside of the root. But loblolly pine, because of its limited aeration system, oxidizes the iron inside the roots. Apparently, as the iron is oxidized inside the root, it complexes with phosphorus forming insoluble compounds. As a result, transport of phosphorus to the shoot is hindered. In soils that are inherently low in phosphorus, most of the phosphorus may be complexed in the root and result in a phosphorus deficiency in the foliage unless phosphorus is added.

When the roots of loblolly pine are placed in waterlogged or anaerobic environments they produce carbon dioxide, ethanol, and malate; end products of anaerobic respiration. Also, the enzyme alcohol dehydrogenase (ADH) becomes more active. The amount of end products formed and ADH activity level depends on the length of time the roots have been subjected to waterlogging stress and on the level of available soil phosphorus. For instance, DeBell et al (1984) found that carbon dioxide, ethanol, and malate production in loblolly pine roots was significantly higher with than without added phosphorus after one growing season in continuously flooded soils.

Phosphorus, also, appears to play an important role in regulating the leaf weight ratio of loblolly pine under a wide range of soil conditions. Hook et al (in prep.) found that the leaf weight ration was low in three waterlogged treatments and high in a well drained treatment without added phosphorus. But with phosphorus the leaf weight ratios were about midway between the extremes and did not vary among treatments. Hence, it seems that under waterlogged conditions phosphorus functions by allocating more of the photoassimilate into stem and foliage and under well drained conditions by allocating more photoassimilate into roots.

Under the stress of soil waterlogging, loblolly pine seedlings exhibit a number of well defined responses. Many of which have been identified previously in other plant species as adaptations to soil waterlogging. Therefore, we have been working under the hypothesis that if performance of families varied under waterlogging stress so would the adaptive responses.

Hence, it should be possible to correlate morphological and physiological responses to family performance. If this is possible, then key adaptations could be identified and used to screen for early indications of waterlogging tolerance among loblolly pine families (see Shear and Hook in these proceedings).

Acknowledgement: This research was supported in part by the USDA, Competitive Research Grant Program, Grant Number 85-08335. We, also, would like to acknowledge the contributions of William H. McKee, Jr., D. S. DeBell, M. R. McKevlin, and J. L. Askew to this research.

Literature Cited

Byram, T. D., W.J. Lowe, C. R. McKinley, J. F. Robinson, A. F. Stauder, and J. P. van Buijtenen. 1984. Thirty-second progress report of the cooperative forest tree improvement program. TX. For. Ser. Circ. 269, 27 p.

DeBell, D. S., D. D. Hook, W. H. McKee, Jr. , and J.L. Askew. 1983. Growth and physiology of loblolly pine roots under various water table level and phosphorus treatments. For. Sci. 30:705-714.

Hook, D. D. and M. R. McKevlin. 1987. Use of oxygen microelectrodes to measure aeration in the roots of intact tree seedlings. In Ecology and Management of Wetlands Vol. 1. Ecology of Wetlands. (eds.) Hook, D. D. and others. pp. 467-476. Croom Helm LTD Publishers. Kent, UK. (in press-expected publication date, August 1987).

Hook, D. D. and S. Denslow. 1986. Variations in physiological responses of four loblolly pine families to two water regimes. In Plant Life in Aquatic and Amphibious Habitats. (ed.) R. M. M. Crawford. British Ecological Society Special Symposium. Blackwell, Oxford.

Hook, D. D. 1984. Adaptation to flooding with fresh water. In Flooding and Plant Growth. (ed.) T. T. Kozlowski pp. 265-294. Academic Press, NY. 356 p.

Hook, D. D., D. S. DeBell, W. H. McKee, Jr., and J. L. Askew. 1983. Responses of loblolly pine (mesophyte) and swamp tupelo (hydrophyte) seedlings to soil flooding and phosphorus. Plant and Soil. 71:387-394.

Hook, D. D. and C. L. Brown. 1973. Root adaptations and relative flood tolerance of five hardwood species. For. Sci. 19:225-229.

Hook, D. D., O. G. Langdon, J. Stubbs, and C. L. Brown. 1970. Effect of water regimes on the survival, growth, and morphology of tupelo seedlings. For. Sci. 16:304-311.

Hook, D. D., W. H. McKee, Jr., and M. R. McKevlin.(in prep.). Relationship of soil phosphorus, water regime, and time on survival, growth, and physiology of loblolly pine.

Kramer, P. J. and T. T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, NY. 811 p.

Mendelssohn. I. A., K. L. McKee, and W. H. Patrick, Jr. 1981. Oxygen deficiency in Spartina alterniflora roots: Metabolic adaptations to anoxia. Sci. 214: 439-441.

McKee, W. H., Jr., and L. P. Wilhite. 1986. Loblolly pine response to bedding and fertilization varies by drainage class on lower Atlantic coastal plain sites. South. J. Appl. For. 10:16-21.

McKee, W. H., Jr., D. D. Hook, D. S. DeBell, and J. L. Askew. 1984. Growth and nutrient status of loblolly pine seedlings in relation to flooding and phosphorus. Soil Sci. Soc. Amer. J. 48:1438-1442.

McKevlin, M. R., D. D. Hook, W. H. McKee, Jr., S. V. Wallace, and J. Woodruff. (accepted). Phosphorus nutrition of flooded loblolly pine seedlings in relationship to iron uptake.(accepted Can. J. For. Res.).

Topa, M. A. and K. W. McLeod. 1986. Effects of anaerobic growth conditions on phosphorus tissue concentrations and absorption rates of southern pine seedlings. Tree Physiol. 2:3270-340.

Topa, M. A. and K. W. McLeod. 1986. Aerenchyma and lenticel formation in pine seedlings: A possible avoidance mechanism to anaerobic growth conditions. Physiol. Plant. 68:540-550.

Vartapetian, B. B. 1987. Ultrastructure studies as a means of evaluating plant tolerance to flooding. In Ecology and Management of Wetlands. Vol. 2. Ecology of Wetlands. (eds.) Hook, D. D. and others. pp. 452-466. Croom Helm LTD Publishers, Kent, UK. (in press-expected publishing date-August 1987).

Webb, J. and W. Armstrong. 1983. The effects of anoxia and carbohydrates on the growth and viability of rice, pea, and pumpkin roots. J. Exp. Bot. 34:579-603.

Williams, C. and F. Bridgewater. 1981. Screening loblolly pine for adaptability to deep peat site: A seedling study of two edaphic seed sources from eastern North Carolina. Proc. Sixteenth South. For. Tree Imp. Conf., Blackburg, VA. May 26-28, 1981.

Yeiser, J. L. and J. L. Paschke. 1987. Regenerating wet sites with bare-root and containerized loblolly pine seedlings. South. J. Appl. For. 11:52-56.

BIOTECHNOLOGY I

MODERATED BY DR. R. J. DINUS

Institute of Paper Chemistry

APPLICATIONS OF BIOTECHNOLOGY IN FOREST TREE IMPROVEMENT

J. W. Hanover[1/]

Abstract.--An assessment of the role of the "new biotechnology" in aiding tree breeders achieve their goal is presented. The array of tools now available include gene and organelle transfer systems, doubled haploids, gene sequencing, restriction fragment length polymorphism, somaclonal variation, *in vitro* screening and others. A model single gene system for 3-carene synthesis is used to illustrate some procedures and problems to be encountered in creating transgenic trees. Finally, a case history examination of a biotechnology firm is presented to show the problems involved in commercializing new technologies.

Additional keywords: Tissue culture, molecular markers, organelle transfer, *in vitro* screening, 3-carene.

INTRODUCTION

Much is being said and written these days about the applications and potentials of the "new" biotechnology in improving agricultural crops and forest trees. Viewpoints among foresters range from skepticism (Libby 1983) through unguarded optimism (Krugman 1985) to apparent fantasy (Durzan 1980). From my perspective, all three viewpoints are appropriate depending upon the time-frame chosen. However, my purpose here is to examine the role of biotechnology in tree improvement within a "practical" time frame that is realistic for tree breeders and future tree improvement practices, over perhaps the next thirty years.

I will focus my discussion on (1) the concept of biotechnology as it relates to forest tree improvement, (2) the array of techniques available to tree breeders now and into the future, (3) a model single gene system for transferring genes in trees to show both problems and opportunities, and (4) a case history examination of a biotechnology firm to illustrate problems in commercializing new technologies.

THE "NEW" BIOTECHNOLOGY

What are we talking about when we use the term "biotechnology?" Imprecision in definitions is one reason why there is considerable confusion and disagreement about the potential for biotechnology in tree improvement.

If we accept the basic definition of biotechnology as the use of living organisms or their components in industrial processes, then all tree breeders

---

1/ Professor, Department of Forestry, Michigan State University, East Lansing, Michigan.

are biotechnologists! So today we refer to our conventional tree improvement practices as the "old" biotechnology. The "new" biotechnology then refers to the novel techniques developed in recent years for altering the genetic composition of plants. These include recombinant DNA techniques, gene and genome transfer methods, and the many tools or techniques associated with genetic manipulation such as cell and tissue culture, molecular markers, in vitro screening and metabolite production, etc. So although all tree breeders may take some comfort in the fact that we are biotechnologists, my discussion and in fact the rest of the world considers only the "new" biotechnology as the biotechnology.

BIOTECHNOLOGY TIMEFRAME

It may be useful at the outset to provide an assessment of what, how and when I believe the various biotechnologies may impact on tree breeding and forest genetics well into the future. This will serve to focus on at least some of the major tools, methods, or goals of biotechnology and how they may benefit tree improvement from my perspective.

Although the tree breeding efforts of the last 40-50 years are now rapidly coming to fruition in terms of commercial use of improved seed, we are still at what must be considered to be a "low" overall level, especially compared with the status of agricultural crop breeding today. With the new biotechnology incentives (funds, tools, and knowledge), tree improvement could rise above its present levels much more quickly than would have been possible without biotechnology. The real danger, however, lies in overemphasizing the new at the expense of the more conventional approaches to tree breeding and genetic analysis. It is absolutely imperative that sound, traditional tree breeding efforts also be accelerated or at lease maintained in order to capture the potential benefits of tree improvement in the foreseeable future. Moreover, the new biotechnologies are heavily dependent on ongoing breeding programs for their success. Unfortunately, there are clear signs that breeding programs are being sacrificed in favor of biotech programs both in forestry and in horticulture (Acuff 1987).

Thus, Figure 1 depicts an increasing level of activity in both tree breeding and forest biotechnology as necessary to raise tree improvement practices from low to high over the next 50 years. At the present time we see a significant stimulation in basic research in forest biology as a result of new commitments to biotechnology. Next on my hierarchy of implementation would be the commercial tissue culture production of select genotypes of important tree species. The role of tissue culture in forest improvement has been clearly outlined by Haissig, et al. (1987). Development of culture systems is important not only for tree production but also as a vehicle for doing the other techniques of genetic manipulation later on. It is interesting to note that it has been more than 50 years since the pioneering tissue culture work of Gautheret (1934, 1940) and Jacquiot (1947, 1949) in France on tree species. In spite of a large research effort on a variety of tree species over these 50 years, we still do not have a commercial system on a forest tree species in the United States. But we may be getting close for such species as loblolly pine, Douglas-fir, Western white pine, and black locust. Nevertheless, the experience with tissue culture serves to illustrate the difference between "potential" and actual "application" for an

important tool of biotechnology.

Other biotechnologies shown in Figure 1 are likewise in the "potential" category and some must in all probability await many years before they are feasible commercially. My estimation of a time frame for each could be substantially out of order, but that is not too important. What is important is that research is being done to varying degrees on each item and basic knowledge is accumulating. This is the key to application no matter how far in the future each is likely to have an impact on our breeding results.

In my view the development of molecular markers such as terpenes, flavonoids, isoenzymes, and DNA restriction fragment length polymorphisms could influence our tree breeding progress before too many years have elapsed. Linkage of these molecular markers with important traits could greatly facilitate selection for both single gene and polygenic traits. They may also be used for strain identification and plant variety protection, as probes for genetic diversity and for constructing genetic maps (Beckmann and Soller 1986).

Whole tree regeneration from single cells will be a key procedure in cell culture allowing the proliferation of genotypes that have been transformed using recombinant DNA, microinjection, or protoplast fusion techniques.

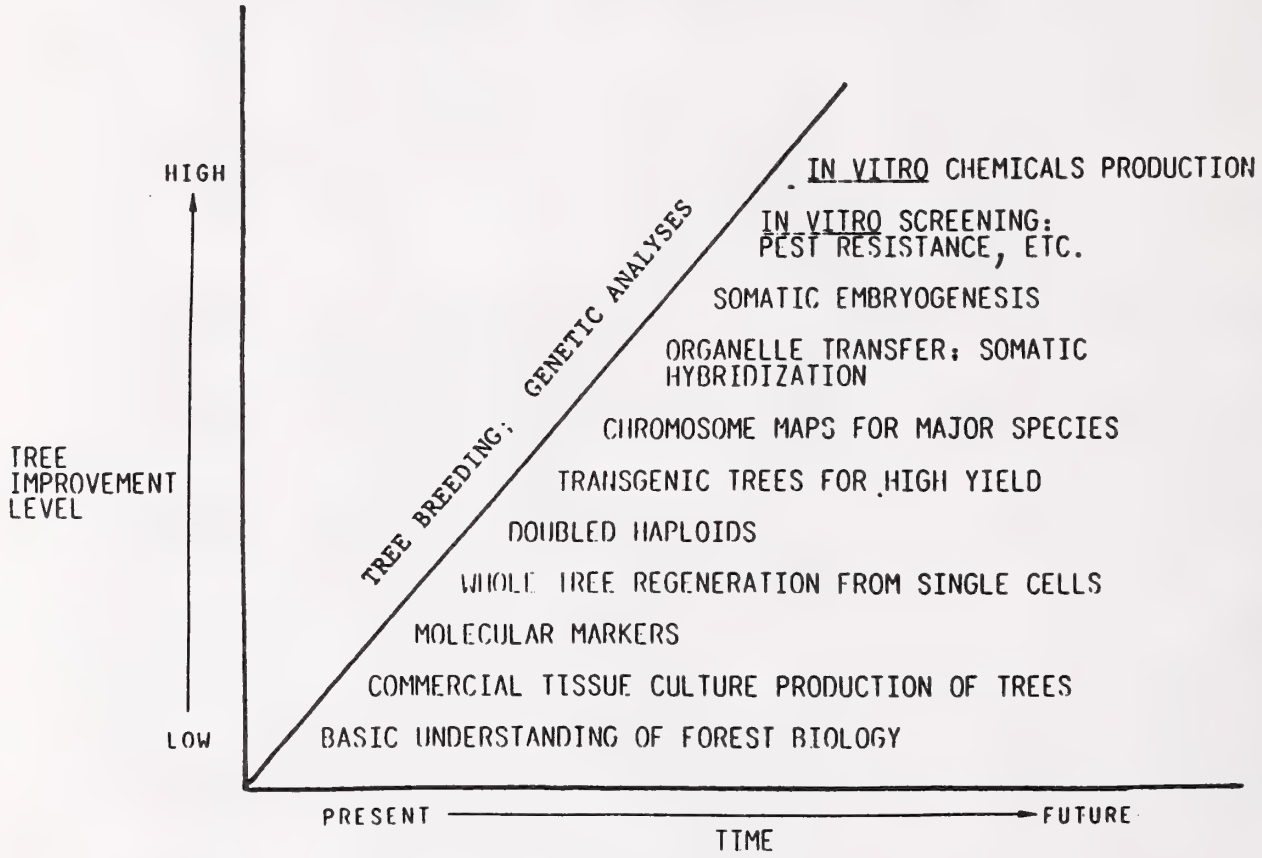
The development of doubled haploids of tree species could be a useful tool in studying gene effects in a homozygous background and perhaps for increasing the efficiency of selection for quantitative traits (Choo 1981). However, many tree species, especially the conifers, may not survive the homozygous state because of an excess genetic load or other genic imbalances, so this procedure may have limited application.

Genetic transformation of trees offers enormous potentials for impacting future tree improvement progress. Methods of genetic manipulation which bypass the sexual process would enable the transfer of specific genes from species to species or tree to tree without the long generation intervals now required. A precedent has already been set for such a feat: the insertion of a gene for tolerance to the herbicide glyphosate into a hybrid poplar has been accomplished (Fillatti et al. 1986). The testing and commercialization of this technique may require many more years of research, but it should serve as a model for other similar approaches where the bacterium Agrobacterium tumefaciens is a suitable vector or where other techniques for incorporating foreign DNA into a recipient cell are available.

I will return to the subject of gene transfer in more detail to illustrate the techniques and problems that are associated with this exciting development which certainly epitomizes the new biotechnology in the minds of most scientists and lay people.

Moving further up the hierarchial scale of Figure 1, I have noted that cytological research and particularly chromosome maps for tree species are needed. At this time I know of no known gene that has been located on the chromosome of a tree. At a time when we are witnessing a gigantic effort to map the entire human genome, it seems appropriate to redirect some effort towards this goal in trees.

FIGURE 1. ASSESSMENT OF THE IMPACTS OF SELECTED BIOTECHNOLOGIES ON FOREST TREE IMPROVEMENT



Cell fusion, in which the DNA complement of organelles such as chloroplasts, mitochondria, or entire protoplasts of contrasting genotypes are enticed to fuse, is another technique for bypassing sex to accomplish somatic hybridization. These methods are fraught with practical difficulties but may have some impact later in the 21st century (Hansen et al. 1986).

Somatic embryogenesis, or the derivation of embryos and thence new trees form somatic tissues, is a tool which could greatly benefit tree improvement in the distant future (El-Nil 1980; Gupta and Durzan 1986, 1987). Presently, occasional embryos have been induced, usually from tissues predisposed to form embryos. Repeatability, consistency, and high frequency regeneration from normal somatic tissue is not possible. If it could be achieved, another method for mass propagation of superior genotypes developed by the tree breeder would be available. Regeneration of trees from single cells or protoplasts is probably even further in the future.

The final two technologies I wish to mention as having potential for benefiting tree improvement depend upon highly sophisticated procedures of tissue culture. If we can develop *in vitro* screening techniques for creating new (somaclonal) variation, or for early evaluation of resistance to pests, such as insects and diseases, or to environmental stresses, such as temperature and drought, the efficiency of selection would increase by orders of magnitude. Such screening techniques would likely depend heavily on detection of chemical genotypes in individual cells, perhaps using diagnostic probes such as poly- or monoclonal antibodies (Haugen and Tainter 1987). This is a difficult task and perhaps far in the future, barring some spectacular breakthrough in the underlying research. Probably equally as difficult as the cell screening technology would be the last technique I will mention which is the actual production of tree products, e.g., high value chemicals, cellulose, perhaps even fiber, *in vitro*. Trees are known for the vast array of diverse chemicals they produce *in vivo*. Yet chemists have barely scratched the surface in describing and developing new substances or processing methods that may have commercial use. Once discovered, high value chemicals could conceivably be produced in large, controlled culture systems with far greater efficiency than in the living tree itself. The cellulose fiber may indeed be the ultimate product of an *in vitro* system. Clearly, a development such as this would drastically alter the tree breeder's protocol!

TRANSGENIC TREES

As stated earlier, the development of methods by which tree breeders could transfer desirable genes directly from one tree to another, thus bypassing the breeding process completely, is a most intriguing concept. In order to show how this might be done and what the major barriers are, I will use a model system, the gene determining 3-carene concentrations in trees. This gene has been well studied (Hanover 1966; Hiltunen 1975; Baradat, et al. 1972) and is recognized as a single gene trait of which there are very few described for tree species. It has proven to be useful in population studies and fingerprinting species and hybrids (Schaefer and Hanover 1986; Bongarten and Hanover 1982). Moreover, 3-carene is a very interesting and potentially useful chemical for other reasons, as shown in Figure 2. For example, there is evidence (Reed, et al. 1986) that 3-carene may be involved in tree resistance to bark beetle attack. If this proves to be the case or if another analogous single gene chemical affecting tree quality is discovered,

FIGURE 2. CHARACTERISTICS OF 3-CARENE

- A UNIQUE MONOTERPENE (ONLY COMMONLY OCCURRING MONOTERPENE WITH CARANE SKELETON)

- CONCENTRATION CONTROLLED BY SINGLE GENE (Cc) IN ALL SPECIES STUDIED. INCOMPLETE DOMINANCE

- GENE EXPRESSED IN SOME TISSUES (BARK, FOLIAGE) AND NOT OTHERS (CONES, XYLEM) DEPENDING UPON SPECIES

- ENVIRONMENTALLY STABLE

- ANTIBIOTIC PROPERTIES

- ALLERGEN PROPERTIES

- IMPLICATED AS INSECT REPELLANT

- ALLELE FREQUENCIES VARY GREATLY BETWEEN SPECIES, POPULATIONS, RACES AND WIND-POLLINATED FAMILIES

then we may wish to transfer such a gene into trees lacking the ability to synthesize the compound of interest. If we choose to transfer the 3-carene gene today, would it be possible and how might it be done? The answer to the first question is no. Therefore, the answer to the second question is based on a number of assumptions that existing barriers to gene transfer can be overcome by future research.

In judging the practicality of transferring a single portion of DNA (gene) from one tree to another, we should be aware of the complexity of gene regulatory controls which must be in place and synchronized with other cell functions in order for new DNA to be expressed when and where we want it to be. Figure 3 provides a concise summary of gene regulation in eukaryotic organisms which is, of course, much more complex than that of the more intensively studies prokaryotes.

On the basis of both facts and theories, I have summarized several procedures that may be employed for transferring the 3-carene gene or another discrete DNA entity from one tree to another (Figure 4). Many of these procedures have been developed on lower organisms or with species that are more easily manipulated than are most woody plants. Nevertheless, progress is being made with trees so that some of the procedures are now feasible even though the whole process is not.

Let me point out some of the major obstacles which prevent us from transferring genes of importance in trees at this time. To begin with, isolation of the enzymes which catalyze synthesis of 3-carene in cells and which are membrane bound will be a formidable task. It is also possible that high 3-carene synthesis is the result of regulatory gene action which would make the isolation of the gene even more difficult. In addition, although the synthesis of oligonucleotides to construct DNA sequences (genes) deduced from protein sequencing is now routine for reasonable small portions of the DNA, creating very large segments may be impossible. Therefore, I opted to go for direct isolation of the mRNA for 3-carene cyclase.

The isolation of messenger RNA is like searching for a needle in a haystack, but theoretically could be done if the 3-carene cyclase can be distinguished from other enzymes catalyzing monoterpene conversions. If sufficient quantities of 3-carene cyclase can be isolated, antibodies to the protein could be produced which would allow synthesis of cDNA which would then be amplified in plasmids and transferred into recipient tissue via the Ti plasmid of Agrobacterium tumefaciens. Alternatively, the plasmids could be inserted by microinjection. Insertion directly by microinjection has not been demonstrated yet. Insertion using Agrobacterium as the vector has been demonstrated only for Populus (Fillatti et al. 1986) as mentioned before but is by no means routine or even feasible for any species at this time. Regardless of which method of insertion is eventually employed, there still remains the task of regenerating the single transformed cell into a new tree. This too is a formidable task and not likely to be possible for many years.

As indicated before, there are many difficulties associated with almost every step outlined here even though most of the basic procedures have been demonstrated for lower organisms. Finally, once the gene is in the recipient tree there is no assurance that: its enzyme product will have the proper substrate, the cell will be able to tolerate the new chemicals, or that it

FIGURE 3. SUMMARY OF GENE REGULATION

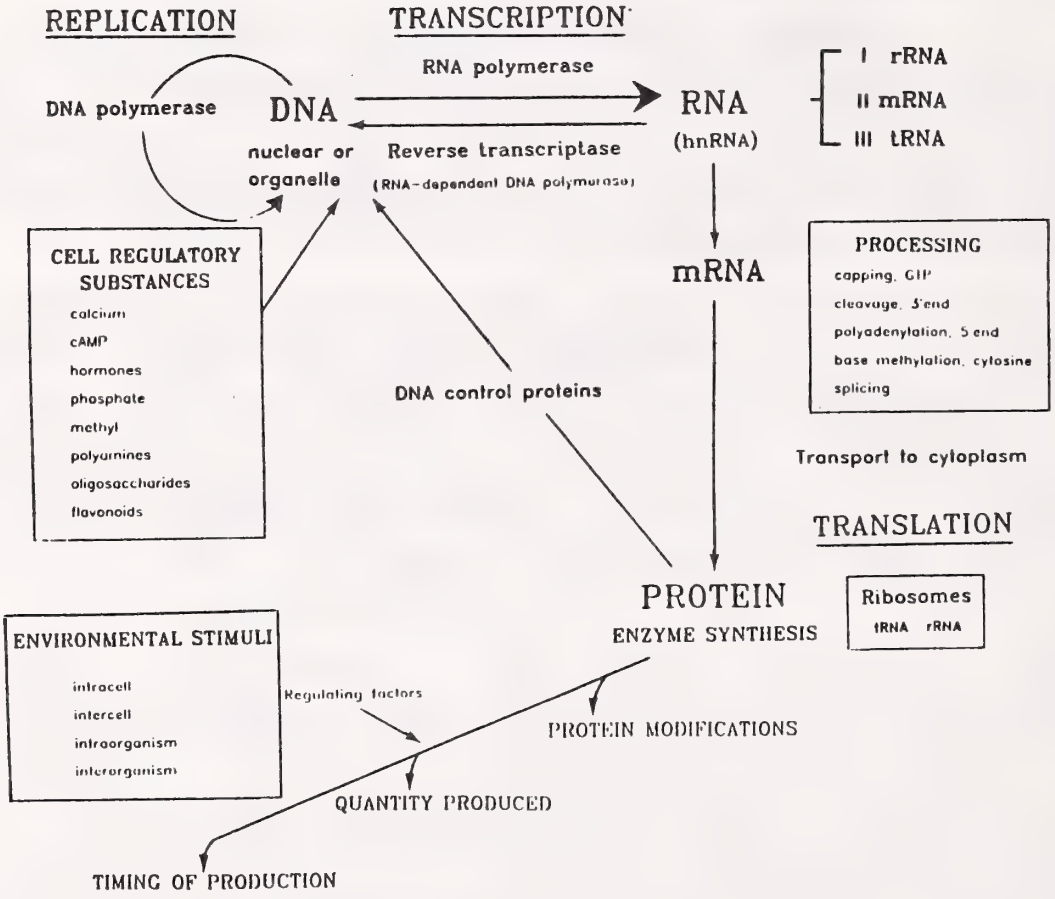
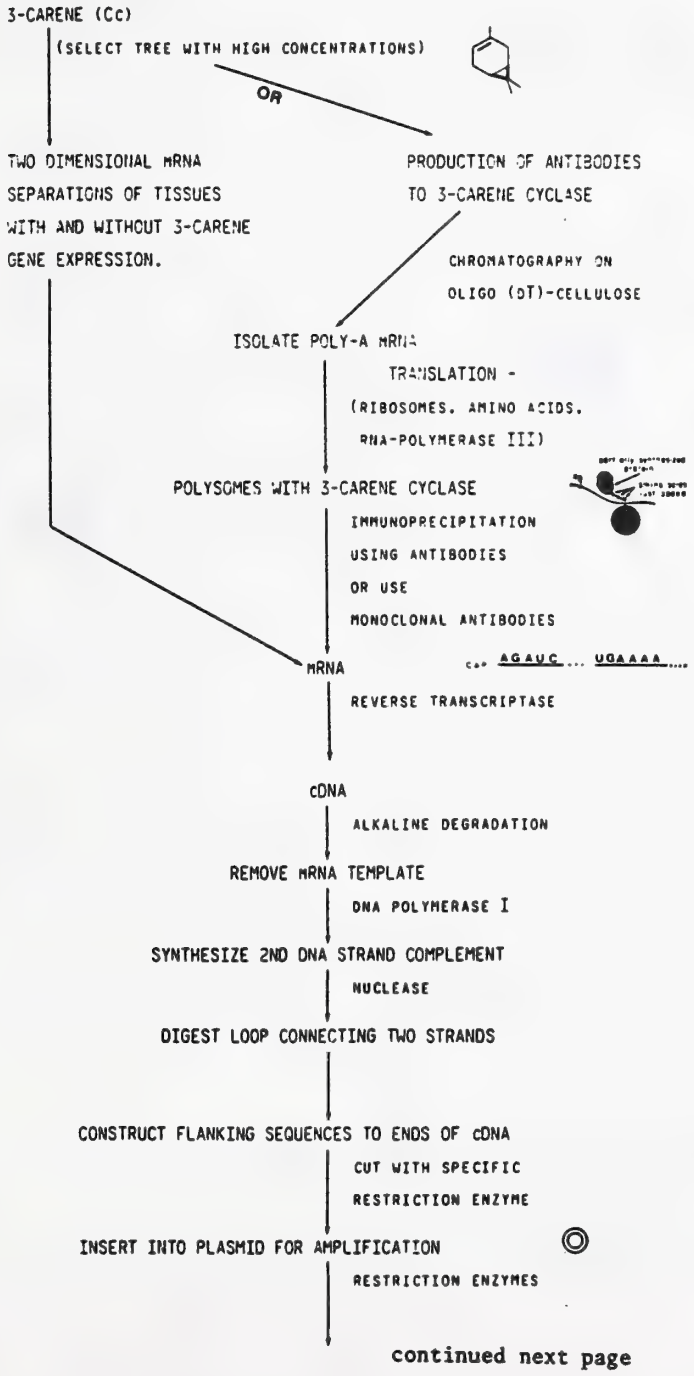
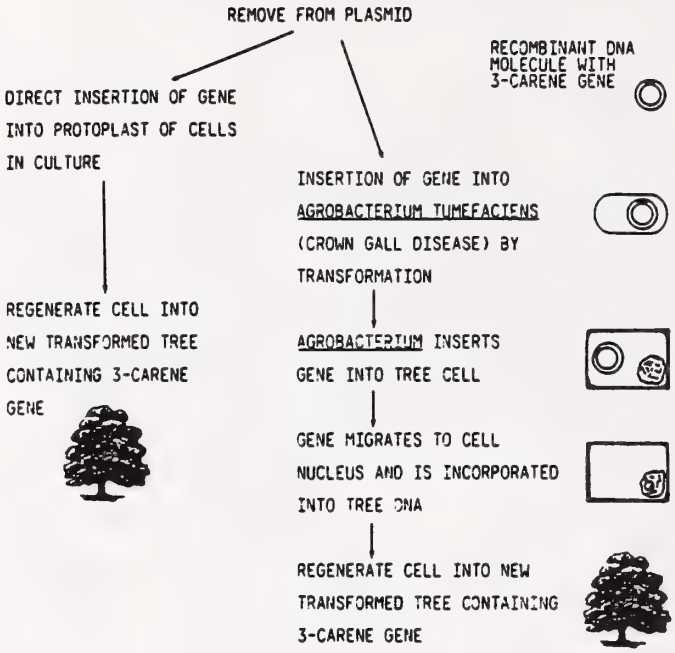


Figure 4. PROCEDURES FOR TRANSFERRING THE 3-CARENE GENE FROM ONE TREE TO ANOTHER


3-CARENE (Cc)
(SELECT TREE WITH HIGH CONCENTRATIONS)
OR
TWO DIMENSIONAL mRNA SEPARATIONS OF TISSUES WITH AND WITHOUT 3-CARENE GENE EXPRESSION.
PRODUCTION OF ANTIBODIES TO 3-CARENE CYCLASE
CHROMATOGRAPHY ON OLIGO (dT)-CELLULOSE
ISOLATE POLY-A mRNA
TRANSLATION - (RIBOSOMES, AMINO ACIDS, RNA-POLYMERASE III)
POLYSOMES WITH 3-CARENE CYCLASE
IMMUNOPRECIPITATION USING ANTIBODIES OR USE MONOCLONAL ANTIBODIES
mRNA
REVERSE TRANSCRIPTASE
cDNA
ALKALINE DEGRADATION
REMOVE mRNA TEMPLATE
DNA POLYMERASE I
SYNTHESIZE 2ND DNA STRAND COMPLEMENT
NUCLEASE
DIGEST LOOP CONNECTING TWO STRANDS
CONSTRUCT FLANKING SEQUENCES TO ENDS OF cDNA
CUT WITH SPECIFIC RESTRICTION ENZYME
INSERT INTO PLASMID FOR AMPLIFICATION
RESTRICTION ENZYMES

continued next page

Figure 4 continued


REMOVE FROM PLASMID
RECOMBINANT DNA MOLECULE WITH 3-CARENE GENE
DIRECT INSERTION OF GENE INTO PROTOPLAST OF CELLS IN CULTURE
INSERTION OF GENE INTO AGROBACTERIUM TUMEFACIENS (CROWN GALL DISEASE) BY TRANSFORMATION
REGENERATE CELL INTO NEW TRANSFORMED TREE CONTAINING 3-CARENE GENE
AGROBACTERIUM INSERTS GENE INTO TREE CELL
GENE MIGRATES TO CELL NUCLEUS AND IS INCORPORATED INTO TREE DNA
REGENERATE CELL INTO NEW TRANSFORMED TREE CONTAINING 3-CARENE GENE

will be properly regulated.

The potentials for gene transfer are great and the possibilities are exciting. Realistically, it may be 50 years before there is a significant impact on practical tree breeding through recombinant DNA technology.

COMMERCIALIZING BIOTECHNOLOGY

The model presented above showed how even the biological feasibility of recombinant DNA technology is probably far from our grasp at this time. Granted, there are some encouraging developments coming from research on several tree species and we are gaining a wealth of basic knowledge about tree biology from the surge of interest in biotechnology. But what are the problems with actual commercialization of the new biotechniques?

To answer this question, I thought it might be informative to take a brief look at an actual situation in commerce today--a case study, if you will. The case is one in which a pioneer biotechnology scientist left the comforts of his University position in 1981 to attempt to commercialize some of the new developments in the fields of tissue culture and genetic engineering. The goal of his company, Crop Genetics International, Inc., was to develop, manufacture and market products that improve crop productivity. Specifically, the company uses cell culture technology to produce "disease free" (not resistant) sugarcane seed products. Also, it is attempting to develop a microbial delivery system for insecticides and fungicides to protect cotton, corn, soybeans, wheat, etc. Crop Genetics International has recently issued a prospectus (February 18, 1987) through Drexel, Burnham, Lambert for the first public sale of common stock to further finance its operations. The principals of the company are competent, dedicated people who are striving hard to bring plant biotechnology into commerce. But the road is not an easy one, judging from the following facts about CGI:

1. Since its formation in 1981 CGI has accumulated a deficit of $7,065,255.
2. CGI has paid no dividends and does not foresee paying any.
3. CGI had sales of seedcane of $964,449 in 1985-86; the costs of production exceeded revenues.

Any new biotechnology company faces a number of risks. These risks for CGI are listed in the prospectus and are undoubtedly typical of those faced by entrepreneurs attempting to capitalize on the new plant biotechnologies. They include:

1. Losses during early development; no assurance of profitability.
2. First sales of seedcane made in 1985-86; cost of production exceed revenues.
3. Company's products are still to be developed, field tested, approved by government agencies, manufactured in production quantities, and marketed successfully.
4. No assurance of product performance.
5. Government regulation; slow and costly process.
6. Potential for product liability; high insurance costs.
7. No assurance of patent protection essential for profitability of products and processes.

8. Highly competitive: other companies may "scoop".
9. High dependence on key personnel who may be mobile.
10. High dependence on universities and research laboratories for appropriate microorganisms.
11. Volatility of sugar prices.
12. Dilution factor: buy shares at $16.00 each; worth $5.53 after offering.

As I stated initially, my purpose here in providing both a model gene transfer scenario and a company case history is not to discourage the pursuit of potentially useful tools of biotechnology but to put these pursuits in their proper perspective in relation to other more conventional tools available to tree breeders, especially natural variation, selection, hybridization, seed production, and cloning. In fact, these tools are even more critical to successful tree improvement today. I am confident that we are indeed in the midst of a revolution in forest genetics and forest biology as well. However, this revolution is not going to be a rapid one in terms of practical applications that tree breeders, seed companies and nurseries can incorporate into their operations in the near future.

ACKNOWLEDGEMENT

The author wishes to thank Daniel Keathley, Michigan State University, for his helpful suggestions in developing portions of this paper.

CELLULAR DROUGHT TOLERANCE STUDIES IN LOBLOLLY PINE

R. J. Newton[1/], J. D. Puryear[2/] and S. Sen[2/]

Abstract.--Drought resistance evaluation and selection in woody, perennial plants often takes several years. Tissue culture technology is being explored as a means of accelerating the selection and tree improvement process and understanding the physiological basis of drought tolerance. The physiological, tolerance response of osmotic adjustment has been demonstrated *in vivo* and *in vitro* in a large number of plants. In loblolly pine (*Pinus taeda* L.), it has been demonstrated only *in vivo*.

The objective was to determine if loblolly pine callus responded *in vitro* with osmotic adjustment when subjected to water stress. Water stress was induced by addition of polyethylene glycol to the media, and the callus water status and solute (potassium, organic acids, sugars and amino acids) content were determined over an eight week period. At a media water potential of -0.7 MPa, stressed callus had a reduced: 1) osmotic potential, 2) solute content, 3) water content and 4) cell volume compared to control, nonstressed callus growing on a media at -0.4 MPa. It was concluded that partial turgor and growth maintenance were accomplished in stressed callus by reduction in cell volume rather than osmotic adjustment. Lack of osmotic adjustment in loblolly pine callus in this study could be attributed to rapid callus dehydration.

Additional keywords: Osmotic adjustment, turgor maintenance, polyethylene glycol (PEG), cell volume.

Cell culture has been used to select for herbicide resistance in tobacco (Chaleff and Parsons 1978) and salt resistance in alfalfa (Croughan et al 1978) *In vitro* selection for drought tolerance is also being explored with grain sorghum (Smith et al 1985), tomato (Bressan et al 1981), and loblolly pine (Newton et al 1985). Callus growth from ten cultivars of grain sorghum subjected to water stress with increasing levels of polyethylene glycol (PEG) added to the medium was highly correlated with field ratings of drought tolerance at the plant level (Smith et al 1985). This suggests that a component of plant drought tolerance may have a cellular basis. Similiarly, correlations between whole plant and cell culture responses have been observed for salt tolerance in grape (Barlass and Skene 1981) and barley (Nabors et al 1980). Therefore, correlations established between cell cultures and whole plants indicate that cell cultures may be effective as an efficient tool for drought tolerance selection.

[1/]Associate Professor and [2/]Research Associate, Dept. of Forest Science, Texas Agricultural Experiment Station, Texas A&M University System, College Station, TX 77843-2135

Cell culture also can be used to observe physiological responses to environmental stress. Osmotic adjustment, a physiological response observed in a variety of whole plants (Morgan 1984), was observed in salt-stressed tobacco cell cultures (Heyser and Nabors 1981) and water stressed tomato cells in culture (Handa et al 1982). Furthermore, genetic variation in osmotic adjustment in whole wheat plants was correlated with yield (Morgan et al 1986), suggesting that osmotic adjustment could be used as a selection criterion in wheat breeding.

Osmotic adjustment has been demonstrated in whole plants of loblolly pine (Hennessey and Dougherty 1984; Seiler and Johnson 1985; Meier and Newton 1987), but it has not been observed in cell culture. If osmotic adjustment could be shown to occur *in vitro* as well as *in vivo* in loblolly pine, this would further substantiate the potential of cell culture (Newton et al 1986) as a technique for drought tolerance selection in tree breeding.

Osmotic adjustment is defined as a net solute accumulation (Jones et al 1981; Morgan, 1984), and it is most often associated with partial or full turgor maintenance (Meyer and Boyer, 1981). Solutes (proline) have been observed to increase in water stressed loblolly pine callus, but the increase was associated with reduced callus growth on media with a water potential below -1.0 MPa, and it appeared that proline was not a significant osmoticum (Newton et al 1986). Solutes which accumulate in most drought stressed plants are low molecular weight products such as carbohydrates, sugar phosphates, organic acids, amino acids and inorganic ions such as potassium and nitrate (Jones et al 1981). It is implied from many studies that osmotic adjustment resides in full or partial turgor maintenance which, in turn, maintains growth, particularly elongation.

The objective of this research was to determine if loblolly pine callus responded with osmotic adjustment when subjected to a moderate water stress (-0.7 MPa). Demonstration of osmotic adjustment was dependent upon the following criteria: 1) callus solute accumulation and 2) a reduction in callus osmotic potential.

MATERIALS AND METHODS

A mixed-orchard source of loblolly pine (*Pinus taeda* L.) seed was obtained from the Texas Forest Service. Excised cotyledons served as an explant source for callus production. Media and culture conditions have been described previously (Newton et al 1986). Callus was produced on Heller supports immersed in MS (Murashige and Skoog 1962) media. Media water potential and callus osmotic potential were determined psychrometrically (Turner et al 1978). Control, nonstress media had a water potential of -0.4 MPa and the stress media had a water potential of -0.7 MPa (10% polyethylene glycol, PEG).

Sugars, amino acids and organic acids were extracted from lyophilized tissues with 80% ethanol (Newton et al 1986). Sugars and organic acids were determined by gas chromatography (Newton et al 1986). Total amino acids were measured spectrophotometrically according to the ninhydrin method of (Rosen 1957). Potassium levels were determined with atomic emission spectroscopy. All growth data are expressed as a mean of ten replicates. Solute data are expressed as a mean of three replicate extractions from 40 mg of lyophilized tissue. Media water potential and callus osmotic potential values are the mean

of 4 sample measurements. Polyethylene glycol associated with callus was measured spectrophotometrically with the trichloroacetic acid method of Lawlor (1970) and modified by Puryear and Newton (unpublished). Polyethylene glycol levels in the tissue were subtracted from the final tissue dry weight; all dry weight data are expressed without the PEG fraction.

Cell volumes were determined with microscopy using an ocular micrometer.

RESULTS AND DISCUSSION

In order to measure the extent of osmotic adjustment in response to water stress, one must determine if: 1) there is a change in osmotic potential, and 2) the change in osmotic potential is due to an accumulation of solutes and/not a loss of water from the callus cells. To test this, callus growing in MS media (-0.4 MPa) for two weeks was transferred to either a new volume of MS media at -0.4 MPa or was placed in MS media with PEG added to obtain a water potential of -0.7 MPa. Callus osmotic potential was measured periodically over an eight week period (Figure 1). An eight week duration was used because this was the length of time previously used to evaluate the water stress performance of calli of several loblolly pine families (Newton et al 1985).

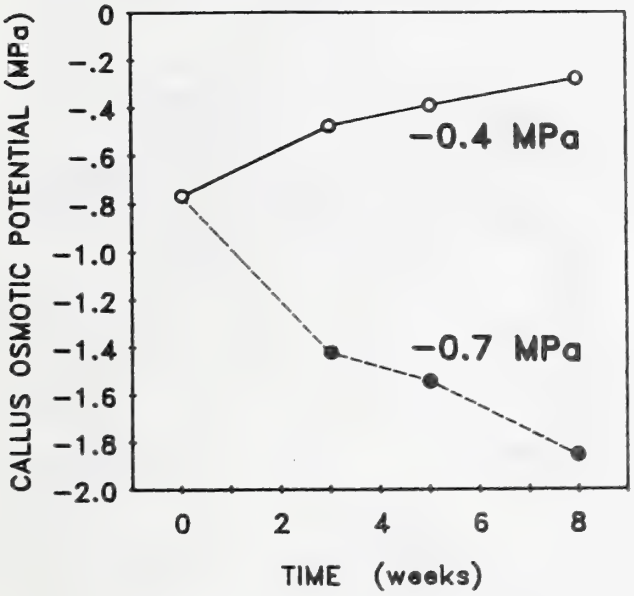


Figure 1. Osmotic potential of nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

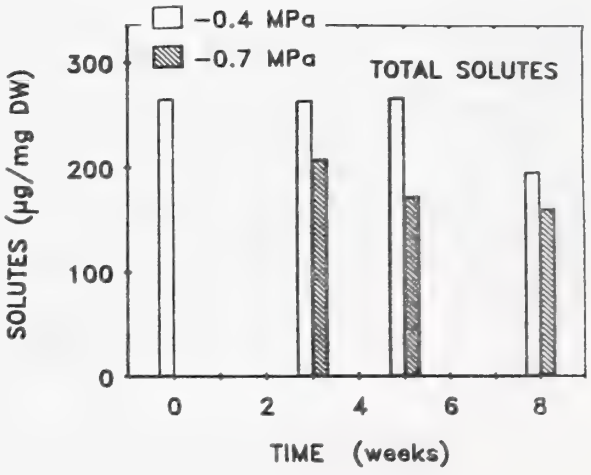


Figure 2. Total solutes (potassium, sugars, amino acids and organic acids) in nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

At the time of transfer, callus osmotic potential was -0.75 MPa (Figure 1). On control media (with a water potential of -0.4 MPa), callus osmotic potential increased to -0.3 MPa by the eighth week. Oppositely, callus transferred to a water stress media (with a water potential of -0.7 MPa), showed a reduction in osmotic potential from -0.75 MPa at week zero to -1.8 MPa after eight weeks (Figure 1).

Callus solute content was then measured to determine its relationship to the callus osmotic potential. In order to determine if there was accumulation, the data were expressed on both a dry weight and a per-callus basis. Common solutes known to accumulate in other plant systems were measured; these were: potassium, sugars, organic acids and amino acids. The total of these four solute fractions was observed to decrease in water-stressed callus (Figure 2 and 3). Therefore, it was apparent that the observed reduction in osmotic potential was not a result of solute accumulation, but was instead due to dehydration in the water stressed callus. This conclusion was verified by analysis of callus growth data.

After eight weeks of growth, fresh weight of callus growing on control media (-0.4 MPa) was over 600 mg while fresh weight on stress media (-0.7 MPa) was less than 100 mg (Figure 4). The initial inoculum callus at the beginning was about 40 mg; therefore, the stressed callus only doubled its fresh weight over the eight week period, whereas the nonstressed callus increased its fresh weight by 15X (Figure 4). Dry weight of the stressed callus was 18 mg and was 30 mg for nonstressed callus (data not shown). This indicated that the fresh

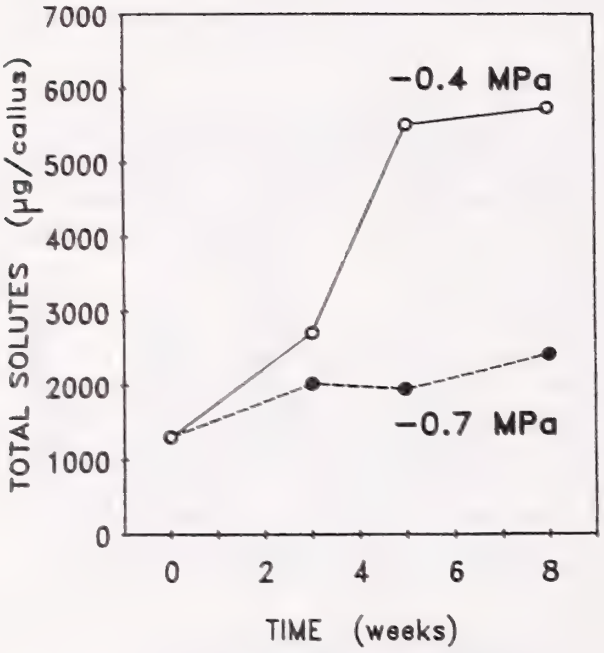


Figure 3. Total solutes (potassium, sugars, amino acids and organic acids) per callus in nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

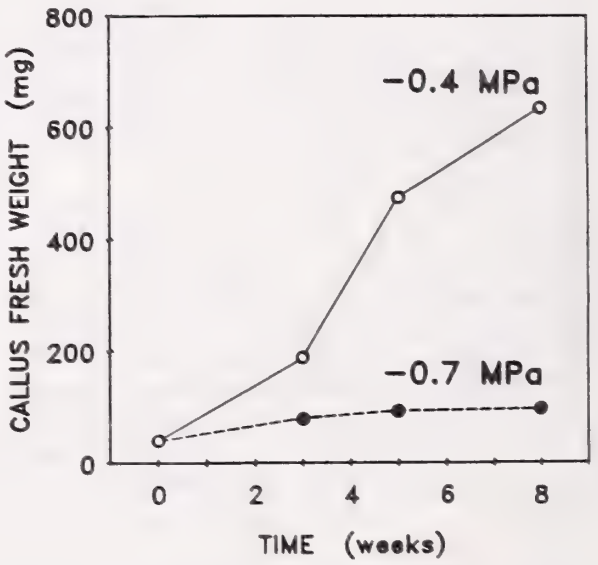


Figure 4. Fresh weight of nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

weight growth difference observed between the contral and stressed callus was due primarily to a reduced water content (fresh weight -- dry weight) in the stressed callus. After eight weeks, the water content of the stressed callus was 80 mg compared to the control callus with a water content of 600 mg.

The reduction in water content of the stressed callus (Figure 4) was also accompanied by a reduction in solute content (Figures 2 and 3) and osmotic potential (Figure 1). The reduced osmotic potential observed was a result of dehydration and not solute accumulation; therefore, there was no osmotic adjustment in the stressed callus. The reduction in total solutes per callus appeared to be a result of reduced callus growth, perhaps a reduction in cell number per callus and/or cell volume.

In contrast, the increase in osmotic potential of nonstressed callus after eight weeks (Figure 1) suggested that cell volumes and/or cell number increased as the fresh weight increased, thus diluting the solute concentration.

Turgor has been shown to be an important component of growth and its total and/or partial maintenance is accomplished by osmotic adjustment (Meyer and Boyer 1981). Since there was no osmotic adjustment observed in stressed callus and there was some maintenance of growth (Figure 4), it is reasonable to assume that at least partial turgor was maintained. In addition to osmotic adjustment, cells can also maintain turgor by reducing their volume (Cutler et al 1977).

The cell population of callus is heterogeneous, ranging from very elongated, rectangular cells to rounded, spherical ones. Both elongated and spherical cell volumes were measured in stressed and nonstressed callus after eight weeks of growth. Cell volumes were reduced 50 to 60% in the stressed callus compared to nonstressed callus (Table 1). This suggested that the callus responded to water stress by producing cells with reduced volumes which perhaps increased the solute concentration and resulted in a reduced osmotic potential. Although cell number/callus was not determined, it is quite likely that it too was reduced when callus was stressed. Therefore if the total solutes/callus (Figure 3) were associated with fewer cells and small cell volumes in response to water stress, the total solute concentration per cell would increase and this would explain the reduction in osmotic potential that was observed (Figure 1). In nonstressed callus, the solute increase (Figure 3) appears to be associated with large cell volumes (and perhaps more cells) and an increase in callus osmotic potential.

Table 1. Cell Volumes of Two Cell Types of Loblolly Pine Callus[1]

Cell Type	Water Potential		Percent Reduction
	-0.4MPa	-0.7MPa	
Spherical	$436 \pm 90 \mu^3$	$164 \pm 66 \mu^3$	62%
Long	$720 \pm 109 \mu^3$	$361 \pm 139 \mu^3$	50%

[1]Callus eight weeks old; n=270

Assessment of turgor maintenance can be accomplished by measuring both callus water potential and osmotic potential and estimating the turgor potential by a difference calculation of these two measurements. We are presently making these measurements in our laboratory in order to estimate turgor. We also are measuring callus turgor with a pressure probe which will be used to corroborate turgor estimates.

The results of this investigation should not be interpreted as to mean that osmotic adjustment does not occur in loblolly pine callus tissue in response to water stress. Under similar experimental conditions, osmotic adjustment was not apparent in stressed callus of a drought tolerant cultivar of sorghum either (Newton et al 1986). Osmotic adjustment is greatly dependent upon the rate of dehydration in the tissues when subjected to water stress (Jones et al 1981). When the water potential of fully expanded sorghum leaves decreased at a rate of over 1.0 MPa/day, no osmotic adjustment occurred, but when the stress rate was 0.1 MPa/day, osmotic adjustment was most evident (Jones and Rawson 1979). It is quite possible that callus mounted on Heller supports is more stressed than indicated by the media water potential in which the support is immersed. If so, rapid dehydration with a rapid decrease in water potential would prevent osmotic adjustment.

ACKNOWLEDGEMENT

This research was supported by the Texas Agricultural Experiment Station, the Texas Forest Service, and the Center for Energy and Mineral Resources, Texas A&M University System, College Station, TX 77843.

LITERATURE CITED

Barlass, M. and K. G. M. Skene. 1981. Relative NaCl tolerances of grapevine cultivars and hybrids *in vitro*. Z. Pflanzen Physiol. Bd. 102:147-156.

Bressan, R. A., P. M. Hasegawa, and A. K. Handa. 1981. Resistance of cultured plant cells resistant to polyethylene glycol induced water stress. Plant Sci. Lett. 21:23-30.

Chaleff, R. S. and M. F. Parsons. 1978. Direct selection *in vitro* for herbicide resistant mutants of *Nicotiona tabaccum*. Proc. Nat. Acad. Sci. USA 75:5104-5107.

Croughan, T., S. Stavarek, and D. W. Rains. 1978. Selection of a NaCl tolerant line of cultured alfalfa cells. Crop Sci. 18:959-963.

Cutler, J. M., D. W. Rains, and R. S. Loomis. 1977. The importance of cell size in the water relations of plants. Physiol. Plant. 40:255-260.

Handa, A. K., R. A. Bresson, S. Handa and P. M. Hasegawa. 1982. Characteristics of cultured tomato cells after prolonged exposure to medium containing polyethylene glycol. Plant Physiol. 69:514-521.

Hennessey, T. C. and T. M. Dougherty. 1984. Characterization of the internal water relation of loblolly pine seedlings in response to nursery cultural treatment: implication for reforestation success. In Seedling Physiology and Reforestation Success. Eds. M. L. Duryea and G. N. Brown. Martinus/Dr. W. Jung Publishers. pp. 225-243.

Heyser, J. W. and M. W. Nabors. 1981. Growth, water content, and solute accumulation of two tobacco cell lines cultured on sodium chloride, dextron and polyethylene glycol. Plant Physiol. 68:1454-1459.

Jones, M. M., N. C. Turner and C. B. Osmond. 1981. Mechanisms of drought resistance. In Physiology and Biochemistry of Drought Resistance in Plants, Eds., L. Paleg, D. Aspinall. Academic Press. Sydney, Aust. pp. 15-37.

Jones, M. M. and H. M. Rawson. 1979. Influence of rate of development of leaf water deficits upon photosynthesis, leaf conductance, water use efficiency, and osmotic potential in sorghum. Physiol. Plant. 45:103-111.

Lawlor, D. W. 1970. Absorption of polyethylene glycols by plants and their effects on plant growth. New Phytol. 69:501-513.

Meier, C. E. and R. J. Newton. 1987. Osmotic adjustment in Pinus taeda L. families in response to water stress. 19th So. For. Tree Impr. Conf. Texas A&M Univ., College Station, TX. June 16-18.

Meyer, R. F. and J. S. Boyer. 1981. Osmoregulation, solute distribution and growth in soyabean seedlings having low water potentials. Planta 151:482-489.

Morgan, J. M. 1984. Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. 35:299-319.

Morgan, J. M., R. A. Hare and R. J. Fletcher. 1986. Genetic variation in osmoregulation in bread and durum wheats and its relationship to grain yield in a range of field environments. Aust. J. Agric. Res. 37:449-457.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-493.

Nabors, M. W., S. E. Gibbs, C. S. Bernstein et al. 1980. NaCl tolerant tobacco plants from cultured cells. Z. Pflanzen Physiol. Bd. 97:13-17.

Newton, R. J., S. Sen, and J. P. van Buijtenen. 1985. Growth changes in loblolly pine (Pinus taeda L.) cell cultures in response to drought stress. Proc. of the 18th For. Tree Impr. Conf. University of So. Miss., Long Beach. May 21-22. pp. 62-64.

Newton, R. J., S. Bhaskaran, J. D. Puryear and R. H. Smith. 1986. Physiological changes in cultured sorghum cells in response to induced water stress. Plant Plysiol. 81:626-629.

Newton, R. J., S. Sen and J. D. Puryear. 1986. Free proline changes in Pinus taeda L. callus in response to drought stress. Tree Physiol. 1:325-332.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. 67:10-15.

Seiler, J. R. and J. D. Johnson. 1985. Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. Forest Sci. 31:742-749.

Smith, R. H., S. Bhaskaran, and F. R. Miller. 1985. Screening for drought tolerance in sorghum using cell culture. In Vitro Cell. Dev. Biol. 21:541-545.

Turner, N. C., J. E. Begg and M. L. Tonnet. 1978. Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at which stomata close. Aust. J. Plant Physiol. 5:597-608.

Chloroplast Ultrastructure of Primary Needles in the Adventitious Shoots of *Pinus taeda*

Jae-Seon Yi and Harry E. Sommer
School of Forest Resources, University of Georgia
Athens, Georgia 30602

ABSTRACT

Chloroplast ultrastructure of loblolly pine was investigated for the primary needles in 3-month-old and 7-month-old adventitious shoots, for those in the seedlings grown for the same length of the time as those shoots, and for 7-month-old brachyblasts. Two media were used to raise the shoots. For ultrastructural studies samples were fixed in 2% glutaraldehyde in 0.2 M sodium cocadylate, post-fixed in 1% buffered osmium tetroxide, dehydrated through an ethanol series, and embedded in Spurr's resin. Sections were stained with 2% saturated uranyl acetate and Reynold's lead citrate. There were no differences in chloroplast ultrastructure due to media. No fundamental differences were recognized in ultrastructure among mature brachyblasts and the primary needles of 7-month-old seedlings or *in vitro* adventitious shoots, while the last one showed the least development of grana. Chloroplasts showing irregular arrangement of lamellar system with or without development of grana were dominant in 3-month-old *in vitro* shoots, while such chloroplasts were rarely seen in the seedlings of the same age.

Key words: *Pinus taeda*, chloroplast ultrastructure, adventitious shoot

INTRODUCTION

In our laboratory we have found and reported several aberrations of chloroplast structure in tissue culture. In sweet gum (*Liquidambar styraciflua*) the normal chloroplast structure is not found in adventitious shoots *in vitro* (Wetzstein and Sommer, 1982). Lee et al. (1985) reported that adventive shoots develop chloroplasts differently from the seedlings under the different light intensity regimes. In slash and longleaf pine callus cultures Birchem et al. (1981) found that chloroplast development was not always complete and that amyloplast-like structures were present instead. Stine (1984) also showed that

chloroplast from adventitious shoots *in vitro* often contained swollen thylakoids, while those from seedlings included such swollen thylakoids only when they had large starch grains, in *Pinus palustris*, *P. elliottii*, and *P. palustris* x *elliottii*. Therefore we consider it important to investigate the ultrastructure of chloroplast in *in vitro* loblolly pine culture.

Chloroplasts, containing chlorophylls and carotenoid pigments, are considered to be one of the most important plant cell organelles, for they are the ultimate suppliers of energy. They are not only sites of photosynthesis; they are also involved in amino acid synthesis and fatty acid synthesis, as well as providing space for the temporary storage of starch.

Studies on the development of plastids in tissue cultures cells have often shown the plastids to resemble those found in quiescent cells (Bornman, 1974). Abberations in the development of plastids in tissues *in vitro* have been reported by Sjoland and Weir (1971) and by Wetzstein and Sommer (1982). Wellburn (1982) reported etiolated pine plants are not photosynthetically competent, although they could form most of the lamellar system in the dark (Oku et al., 1977), which differs from the angiosperms that cannot synthesize chloroplasts in the dark.

Exogenous kinetin in bean leaf culture increased the number of grana per chloroplast and the size of chloroplasts (Naito et al., 1981; Tsuji et al., 1979). Some tobacco suspension cultures were capable of differentiation of chloroplasts only with the application of a cytokinin (Axelos and Peaund-Lenoel, 1980; Seyer et al., 1975). In Sycamore maple (*Acer pseudoplatanus* L.) callus it was reported by Sunderland (1966) that NAA and 2,4-D inhibited chlorophyll formation. It was reported

that it seemed chloroplast differentiation was correlated to the differentiation of callus into organs (Barnett, 1978). He found amyloplasts in undifferentiated callus but chloroplasts in stem-like organs originated from callus in Monterey pine. Simola (1982) reported the well-organized chloroplasts were found in the needle-like structures in the callus cultures of Norway spruce.

Pinus taeda embryos formed adventitious shoots in vitro but showed much variation in shoot establishment ability and vigor, depending on medium and exogenous hormone dosages (unpublished data). Electron microscopic studies were done to determine whether there is a difference in chloroplast structure between primary needles from adventitious shoots, primary needles from seedlings, and brachyblasts.

The overall purpose of this study is to compare the chloroplast ultrastructure for different levels of growth regulators and for medium formula.

However, at this time we will report only on the ultrastructure of chloroplasts in adventitious shoots induced on MGE, a medium modified from Gamborg and Eveleigh (1968) and on MLM, a medium modified from Lloyd and McCown (1981), each of which contained 5 mg/l 6-benzylamino-purine (BAP) and 0.1 mg/l naphthaleneacetic acid (NAA).

Throughout this paper "seedling" stands for a plant with or without a root system formed by the growth of the apical meristem and "shoot" for the analogous structure formed by adventive organogenesis on cotyledons of the embryo, if no additional detail is mentioned.

MATERIALS AND METHODS

Embryos were cultured 3 to 4 weeks for initiation of buds and transferred for the growth of buds to the non-hormonal charcoal medium reduced by half in major and minor salts except iron. Explants were subcultured every 3 to 5 weeks.

The second subculture media included no charcoal. All media contained sucrose and agar at 2% and 6%, respectively. All cultures were placed in the growth room at 23 to 27 centigrade. Illumination was given by cool white fluorescent lamps with an intensity of approximately 1506+540 lux at the level of the explant with a 16-hour photoperiod.

Primary needles from 3-month-old and 7-month-old adventitious shoots were harvested. In addition to 7-month-old brachyblasts, primary needles from the seedlings germinated and grown under the same *in vitro* cultural environment were used for comparison.

All 7-month-old needles were fixed and embedded in November, 1986 for this investigation. Others were fixed and embedded in May, 1987.

Methods for fixation, embedding, and staining are as follows.

FIXATION

1. 1-mm-long sections were cut from the middle part of the needles and immediately fixed in 2% glutaraldehyde in 0.2 M sodium cocadylate buffer, *in vacuo* under the room temperature for 12 hrs.

2. Sections were washed 5 mins two times, 30 mins once, and 10 mins once with 0.2 M sodium cocadylate buffer, and then post-fixed for 4 hrs *in vacuo* at 1-4 C in 1% OsO_4 in 0.2 M sodium cocadylate buffer.

3. Sections were washed in the same buffer for 10 mins, followed by a 30 min wash, and finally an additional 10 min wash.

4. The samples were dehydrated using a graded ethanol series by placing them for 15 mins sequentially in 30, 50, 70, 80, and 95% ethanol, and 100% ethanol twice.

EMBEDDING

1. Infiltration was done successively for 4 hrs, 3.5 hrs and 7 hrs, in each of 1:3, 1:1, and 3:1 Spurr's resin (1969): 100% ethanol *in vacuo*.

2. The embedment media were changed with pure Spurr's resin for 4, 6, and 4 hrs *in vacuo*.

3. The samples were placed into oven-dry embedding capsules with fresh resin and polymerized for 12 to 24 hrs at 70 C.

TRIMMING AND SECTIONING

The sample blocks were trimmed to form a trapezoid through the middle of the sample. Thin sections were made on a Sorval MT-2 ultramicrotome using a diamond knife.

One micron-thick sections were made for the examination with a light microscope after staining with Paragon 1301 stain (Spurlock et al., 1966). The sections were spread with chloroform vapor prior to being picked up on formvar coated copper grids.

STAINING

The sections were stained for 15 mins with 2% aqueous uranyl acetate, and then rinsed 4 times for 15 secs each with distilled water. Rinsed specimens were stained again with Reynolds lead citrate (Reynolds 1963) for 10 mins in the covered petri dishes free from carbonate by NaOH pellets, followed by 4 rinses of distilled water 15 sec each.

Prepared specimens were examined using Philips 400 transmission electron microscope. The accelerating voltage was set at 80 KV.

RESULTS AND DISCUSSION

Plastids, in addition to cell walls and vacuoles, are specific components of plant cells. They are bounded by an envelope, a pair of unit membranes, and consists of a lamellar system and a matrix substance, stroma, which includes plastid DNA, ribosomes, and occasionally starch grains. Mature plastids are classified on the basis of the kinds of pigments they contain (Whatley et al., 1978). They are etioplast, amyloplast, chromoplast, and chloroplast.

Chloroplasts are usually found disk-shaped with their long axes parallel to the cell wall. The stroma is traversed by an elaborate system of membranes in the form of flattened sacs, thylakoids. A granum is formed when stroma thylakoids organize a structure like a stack of coins. These characterize chloroplasts. The grana are connected with each other by stroma thylakoid. The thylakoid structure is similar to the plastid envelope in that it consists of two unit membranes. The chloroplasts of 7-month-old brachyblast of loblolly pine are shown in Fig. 2. Well-developed grana are conspicuous (Fig. 3). Other cell

organelles in addition to chloroplast are shown in Fig. 1. Starch grains and osmiophilic globules are often found in brachyblast chloroplasts. In Fig. 3 chloroplast ultrastructure is well preserved, and shown are grana, large starch grain, and osmophilic globules.

The chloroplast envelope is also clearly visible. Chlorophylls and carotenoid pigments, which are responsible for photosynthesis, are found embedded in thylakoid membranes (Mohr, 1981).

Chloroplasts in the primary needles of 3-month-old plants are shown in Figs. 4 and 5. Many embryos cultured on MLM produced seedling by the growth of the apical meristem in addition to the adventitious shoots on cotyledons. The primary needles of these seedlings and shoots were recommended materials to investigate chloroplast development.

Fig. 4 shows the fine structure of the primary needle chloroplast in the seedling originated from an embryo which was cultured on MLM including 5 mg/l BAP and 0.1 mg/l NAA. Chloroplasts similar to this were found in the seedlings germinated and grown on either MLM or MGE without growth regulators.

In Fig. 5 is shown a chloroplast in the primary needles of 3-month-old adventitious shoots on the cotyledons from the same embryo that produced the seedling mentioned in Fig. 4. Stroma thylakoids are arranged loosely and irregularly. It is not expected that the seedling and the adventitious shoot originating from the same embryo and grown under the same in vitro conditions would show the ultrastructural differences in chloroplasts.

These differences are supported by Figs. 6 and 7. Fig. 6 is the chloroplast in 7-month-old seedling grown on MGE without growth regulators. Fig. 7 is a highly magnified chloroplast in the shoots of

the same age as that in Fig. 6. These were not differentiated from the same embryo as those in Figs. 4 and 5. In the shoots grana were not as well developed as those in the seedlings. Swollen thylakoids were often found that were rare in the seedlings (Fig. 7). Stine (1984) also showed the similar ultrastructural differences in several pine species.

After the growth of 4 months, chloroplasts developed significantly (Figs. 5 and 7), even though the grana with many stacks of thylakoids were not frequent and some swollen loculi were still present. Even the 3-month-old seedling had formed the grana of up to 12 stacks of thylakoids (Fig. 4).

In sweetgum adventitious shoots, chloroplasts with irregular lamellar system were also reported when grown under various light intensity regimes (Lee et al., 1985). They also found large starch grains in the seedlings but not in the plantlets. However, they did not determine if starch accumulation was cyclic, or whether seedlings and cultured plantlets differed in this respect. Starch grains were shown in 7-month-old adventitious shoots (Fig. 8). The seedlings of this age showed the development of starch grains, but none of 3-month-old seedlings or shoots showed these.

Many in vitro factors, such as kinetin and NAA, affected the chloroplast development (Naito et al., 1981; Tsuji et al., 1979). In some tobacco cultures chloroplasts differentiated only when exogenous cytokinin was applied (Seyer et al., 1975; Axelos and Peaund-Lenoel, 1980). Further studies are required to investigate the effects of growth regulators on the pine chloroplast development in the in vitro shoots. But it seems that there is no significant difference in the chloroplast ultrastructure between two media, MGE and MLM.

LITERATURE CITED

Axelos, M., and C. Peaund-Lenoel. 1980. The apoprotein of the light-harvesting chlorophyll a/b complex of tobacco cells as a molecular marker of cytokinin activity. Plant Sci. Lett. 19;33-41.

Barnett, J. R. 1978. Fine structure of parenchymatous and differentiated Pinus radiata callus. Ann. Bot. 42;367-373.

Birchem, R., H. E. Sommer, and C. L. Brown. 1981. Comparison of plastids and pigments of Pinus palustris Mill. and Pinus elliottii Engelm. in callus tissue culture. Z. Pflanzenphysiol. 102(2);101-107.

Bornman, C. H. 1974. Cytodifferentiation in tissue culture. In tissue culture and plant science (H. E. Street ed.), 43-70. Academic Press, New York. 502p.

Gamborg, O. L., and D. E. Eveleigh. 1968. Culture methods and detection of glucanases in suspension culture of wheat and barley. Can. J. Biochem. 46;417-421.

Lee, N., H. Y. Wetzstein, and H. E. Sommer. 1985. Effects of quantum flux density on photosynthesis and chloroplast ultrastructure in tissue-cultured plantlets and seedlings of Liquidambar styraciflua L. towards improved acclimatization and field survival. Plant Physiol. 78;637-641.

Lloyd, G., and B. McCown. 1981. Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Comb. Proc. Intern. Plant. Prop. Soc. 30;421-427.

Mohr, M. 1981. Control of chloroplast development by light, some recent aspects. In Photosynthesis V. Chloroplast development (G. Akoyunoglou ed.), 869-883. Balaban, Philadelphia. 1018p.

Naito, K., K. Ueda, and H. Tsuji. 1981. Differential effects of benzyladenine on the ultrastructure of chloroplasts in intact bean leaves according to their age. Protoplasma. 105;293-306.

Oku, T., S. Furuta, K. Shimada, Y. Kobayashii, T. Ogawa, Y. Inoue, and K. Shibata. 1977. Development of photosynthetic apparatus in spruce leaves. In Photosynthetic organelles, special issue of Plant and Cell Physiol. 437-444.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17; 208-212.

Seyer, P., D. Marty, A. M. Lecure, and C. Peaund-Lenoel. 1975. Effect of cytokinin on chloroplastic differentiation in cultured tobacco cells. Cell Differ. 4;187-197.

Simola, L. K. 1982. Ultrastructure of callus cultures from Betula pendula and Picea abies. In Plant tissue culture 1982 (A. Fujiwara ed.), 173-174. Maruzan, Tokyo. 839p.

Sjoland, R. D., and T. E. Weir. 1971. An ultrastructural study of chloroplast structure and differentiation in tissue cultures of Streptanthus tortuosus (Cruciferae). Amer. J. Bot. 58;172-181.

Spurlock, B. O., M. S. Skinner, and A. A. Kattine. 1966. A simple rapid method for staining epoxy-embedded specimens for light microscopy with the polychromatic stain paragon-1301. Amer. J. Clin. Path. 46-252.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrast. Res. 26;31-43.

Stine, M. 1984. Adventitious shoot formation on embryos of Pinus palustris Mill., P. elliottii Engelm., and P. palustris x elliottii. 112p. M. S. thesis, Univ. of Georgia, Athens, GA.

Sunderland, N. 1966. Pigmented plant tissue in culture. I. Auxins and pigmentation in chlorophyllous tissues. Ann. Bot. 30;253-268.

Tsuji, H., K. Naito, and I. Hatakeyama. 1979. Benzyladenine-induced increases in DNA content per cell, chloroplast size, and chloroplast number per cell in intact bean leaves. J. Expt. Bot. 30;1145-1151.

Wellburn, A. R. 1982. Bioenergetic and ultrastructural changes associated with chloroplast development. Int. Rev. Cyt. 80;134-189.

Wetzstein, H. Y., and H. E. Sommer. 1982. Leaf anatomy of tissue-cultured Liquidambar styraciflua (Hamamelidaceae) during acclimatization. Amer. J. Bot. 69;1579-1586.

Whatley, J. M. 1978. A suggested cycle of plastid developmental interrelationships. New Physiol. 80;489-502.

Figs. 1 to 8. Mesophyll cells and chloroplasts in loblolly pine needles. c, cytoplasm; ce, chloroplast envelope; cp, chloroplast; cw, cell wall; g, granum; is, intercellular space; m, mitochondrion; o, osmophilic globule; s, stroma; sg, starch grain; t, thylakoid; v, vacuole.

Fig. 1. General structure of mesophyll cell in brachyblast.

Fig. 2. Detail of chloroplast in brachyblast. Chloroplast with a large starch grain and well-developed grana.

Fig. 3. Higher magnification of brachyblast chloroplast.

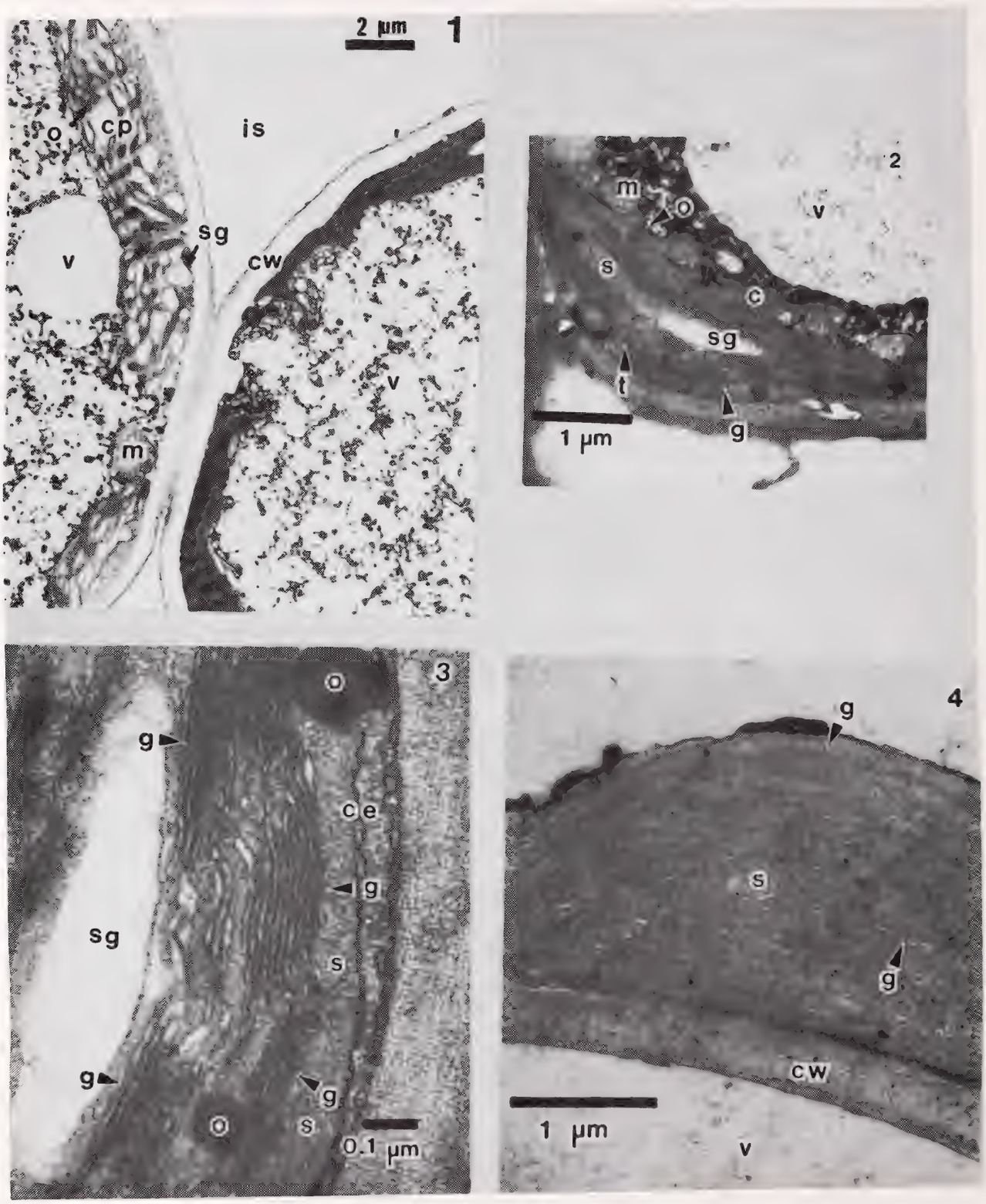
Fig. 4. Chloroplast of primary needle in the 3-month-old seedling originated from an embryo grown on MLM containing 5 mg/l BAP and 0.1 mg/l NAA. Well-arranged lamellar system.

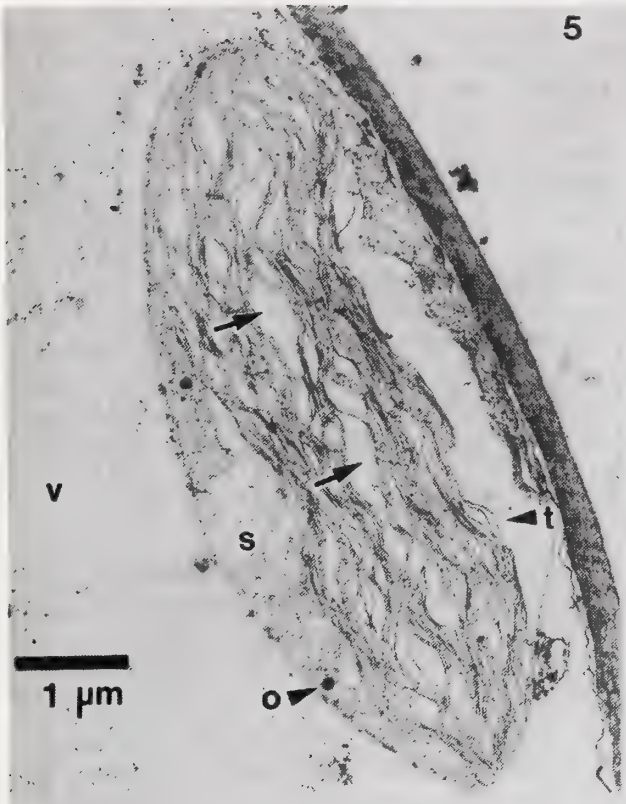
Fig. 5. Chloroplast of primary needle in the 3-month-old adventitious shoot induced on the cotyledon of the same embryo mentioned in Fig. 4. Irregular arrangement of lamellar system.

Fig. 6. Chloroplast of primary needle from the 7-month-old *in vitro* seedling germinated and grown on MGE without growth regulators.

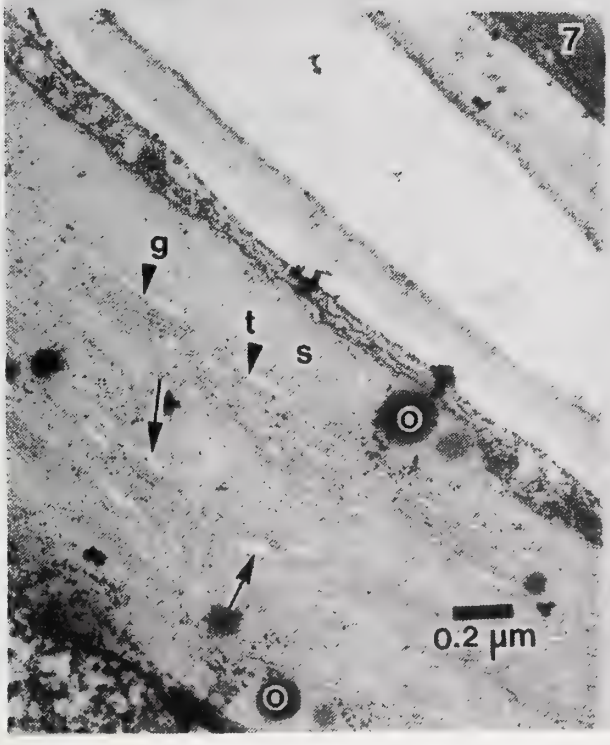
Fig. 7. Chloroplast of primary needle from the 7-month-old adventitious shoot induced on MGE containing 5 mg/l BAP and 0.1 mg/l NAA. Well-organized grana but some swollen loculi.

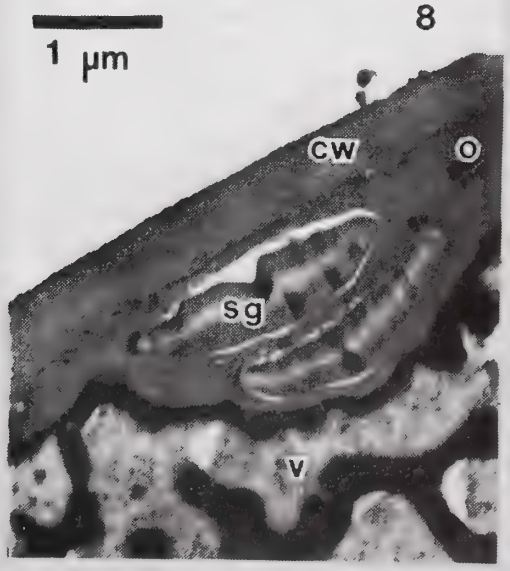
Fig. 8. Chloroplast containing large starch grains in the 7-month-old *in vitro* shoot.


2 µm
1
o
cp
is
sg
v
cw
v
m
2
m
o
v
s
c
sg
t
g
1 µm
3
o
g
ce
g
sg
s
g
g
o
s
0.1 µm
4
g
s
g
cw
1 µm
v


5
v
s
t
o
1 µm

1µm
6
s
g
g
o


7
g
t
s
o
0.2 µm
o


1 µm
8
cw
o
sg
v

THE FREQUENCY OF PLANT REGENERATION FROM NORWAY SPRUCE SOMATIC EMBRYOS

M. R. Becwar[1], S. A. Verhagen[2], and S. R. Wann[3]

ABSTRACT

Regeneration via somatic embryogenesis holds much promise as a system for mass propagation of genetically improved and/or engineered conifers. Most reports to date on conifer somatic embryogenesis have described initiation of embryogenic callus and in some cases have verified the developmental potential of the somatic embryos. There is little quantitative information on the frequency at which conifer somatic embryos undergo three critical stages of development: 1) the maturation of somatic proembryos to the cotyledonary stage, 2) germination or primary root development, and 3) plant survival (conversion) and growth in soil. This report describes methods for and success rates in stimulating development at each of these stages for somatic embryos derived from immature and mature seed embryos of Norway spruce. Results indicate that the somatic embryo maturation process remains the most difficult and least efficient. Germination frequencies as high as 82% have been achieved, and results concerning efficiency of plant survival in soil are presented. Approaches to improve the "rate-limiting" maturation step are also discussed.

Additional Keywords: Picea abies, somatic embryogenesis

INTRODUCTION

Somatic embryogenesis is the production of embryos vegetatively under test-tube or in vitro conditions. This process holds much promise as a clonal system for mass propagation of plants because the production of embryos is via mitotic division of vegetative cells, rather than the sexual fusion of gametic cells. Furthermore, the process is amenable to automation. Lastly, because the somatic embryos produced in culture are analogs to seed embryos, it is possible to encapsulate them and form artificial seeds (Redenbaugh et al. 1986). Such somatic seed would interface well with existing nursery production systems in conifers.

---

[1]Industrial Research Fellow, Westvaco Corp., Summerville, SC and Adjunct Assistant Professor, The Institute of Paper Chemistry, Appleton, WI.
[2]Research Fellow, The Institute of Paper Chemistry, Appleton, WI.
[3]Industrial Research Fellow, Union Camp Corp., Princeton, NJ and Adjunct Assistant Professor, The Institute of Paper Chemistry, Appleton, WI. The authors express sincere appreciation to Debbie Hanson, Lynn Kroll, and Judy Wyckoff, Gary Wyckoff, Robert Arvey, and Egon Hummenberger for their assistance in these studies.

The first reproducible report of somatic embryogenesis in conifers was with Norway spruce (Hakman et al. 1985). Reports of somatic embryogenesis in European larch (Nagmani and Bonga 1985), Radiata pine (Smith et al. 1985), Sugar pine (Gupta and Durzan 1986), loblolly pine (Gupta and Durzan 1987), and white and black spruce (Hakman and Fowlke 1987, Nagmani et al. 1987) have followed recently. In order for somatic embryogenesis to be developed commercially, the process needs to be efficient and repeatable. There is little quantitative information on the frequency at which conifer somatic embryos undergo three stages of development: maturation of immature embryos, emergence and growth of the root ("germination"), and establishment and growth in soil (conversion). We report here results on efficiencies of these stages in establishing somatic embryo plants of Norway spruce. The embryogenic callus used in these studies was initiated from embryos of immature and mature (stored) seed. This is also the first demonstration of overwintering and subsequent spring growth flush of conifer somatic embryo plants.

MATERIALS AND METHODS

Initiation Embryogenic callus was initiated from immature embryos of an open pollinated Norway spruce tree located in Appleton, Wis. in July, 1985 as previously described (Becwar et al. 1987a). A modified MS basal medium (von Arnold and Erickson 1981) supplemented with 2,4-D (2 mg/l) and BA (1 mg/l), hereafter refered to as HM, was used. Embryogenic callus was also initiated from mature embryos of seed purchased from Quality Tree Seed Inc., Brewster, N.Y. The seeds were surface sterilized in 30% H_2O_2 for 45 min., rinsed 3 times, and imbibed overnight prior to excision of the embryos. Initiation from mature embryos was on HM and BLG (Amerson et al. 1985), a modified MS medium which replaces the NH_4NO_3 with 5 mM glutamine. The BLG was supplemented with either NAA or 2,4-D (2 mg/L) and BA (1 mg/L). All cultures were maintained at 23°C with 16 hr irradiance (15-50 $\mu E\ m^{-2}\ s^{-1}$) from cool-white fluorescent and incandescent lights.

Maturation and Germination To obtain somatic embryo development, calli were transferred to basal medium with activated charcoal (1%) lacking growth regulators for seven days, and then to basal medium with IBA and ABA (1 µM each) (Becwar et al. 1987a). In one experiment buthionine sulfoximine (BSO) was added to the above developmental medium to test its effect on maturation (Wann et al. 1986). Individual somatic embryos were removed from the calli when cotyledons appeared distinct and green and hypocotyl elongation had occurred. Maturation frequencies were determined based on the fraction of immature somatic embryos, counted after 21 days on the development medium (Becwar et al. 1987b), which developed to the cotyledonary stage. Three techniques were tested for somatic embryo germination on quarter strength HM medium: 1) radicle of embryo in medium (control), 2) radicle of embryo on medium surface (slant), and 3) cotyledons of embryo in medium with culture vessel inverted (hanging).

Conversion Two experiments were conducted on transferring somatic embryo plantlets, with primary roots of approximately 1 cm, to soil. In the first trial somatic plantlets and zygotic seedlings at an equivalent stage of development were transferred to a Jiffy soil mix in Magenta GA7-3 plastic vessels.

These somatic and zygotic plantlets were grown in the growth chamber as previously described for two months before transfer to the greenhouse. High relative humidity was maintained by controlling the tightness of the GA7-3 vessel lids. In the second trial somatic plantlets were transferred directly from in vitro to greenhouse conditions in Spencer-Lemaire "book planters" containing soil (5:1, Jiffy soil mix:Perlite). High relative humidity was maintained by enclosing the book planter trays in clear plastic bags. All greenhouse plants were fertilized with osmocote (14-14-14) and grown under ambient light supplemented with fluorescent light (16 hr, 100 $\mu E\ m^{-2}\ s^{-1}$) at 20-25°C. Plants which set resting terminal buds were transferred to lower temperatures (5-15°C) and ambient light for 7 weeks. Plants overwintered at -5 to 10°C. On March 1 plants were returned to ambient greenhouse conditions (20-25°C).

Data Analysis Data are presented as mean values among replicated experimental units (e.g., culture plates) and analyzed by ANOVA followed by Duncan's New Multiple Range Test for multiple comparisons of means. Mean values followed by unlike letter are significantly different (P = 0.05).

RESULTS AND DISCUSSION

Initiation Embryogenic callus lines were derived from individual embryo explants. Over 75% of the immature embryo explants collected in early July in Wisconsin initiated embryogenic callus (Becwar, et al. 1987a). Initiation of the desirable embryogenic callus on the HM basal medium dropped off rapidly with time, and by the end of July only a nonembryogenic callus was initiated.

We have been able to extend the window for initiation of embryogenic callus in Norway spruce to mature seed by utilizing a modified basal medium (Table 1). Specifically, by culturing mature embryos on half-strength BLG with either 2,4-D or NAA as auxins, from 21 to 27% of the explants initiated embryogenic callus and over half of the lines were successfully maintained for over four months. Although it was possible to initiate embryogenic callus at very low frequencies from mature embyros cultured on HM, none of these lines could be maintained (Table 1). Furthermore, the change from full strength HM to full strength BLG did not result in initiation from mature embyros. These results point out that levels of other components of the BLG medium, besides absence of ammoniacal nitrogen, are essential for extending the initiation window to mature embryos. We have verified that the somatic embryos initiated on 1/2 BLG develop to cotyledonary stage (Fig. 1A-C) and germinate (Fig. 1D).

Table 1. Effect of modifications in the medium on initiation and maintenance of embryogenic callus from mature embryos of Norway spruce.

Basal medium	Auxin/Cytokinin (mg/L)		Number of explants	Embryogenic callus (% explants)	
	2,4-D/BA	NAA/BA		4-6 weeks	4-5 months
HM	2/1	---	158	3	0
1/2HM	---	2/1	30	0	0
BLG	---	2/1	50	0	0
1/2BLG	2/1	---	40	21	11
1/2BLG	---	2/1	120	27	16

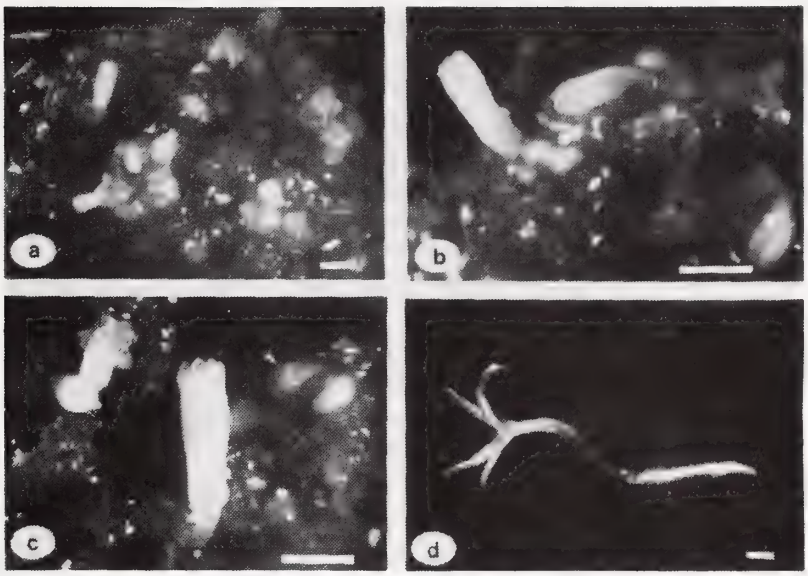


Figure 1. Plantlet regeneration from embryogenic callus initiated from mature embryos of Norway spruce. A: Individual embryos visible after 21 days on development medium. B & C: Maturation of somatic embyros, hypocotyl elongation and cotyledonary development. D: Germination, primary root growth. Scale bars = 1 mm.

Maturation The frequency at which developing somatic embryos reached the mature (cotyledonary) stage is summarized in Table 2. Note that there were differences in maturation frequencies among embryogenic callus lines. Line (NS1)5 had maturation frequencies of 1 to 4%, whereas lines (NS1)8 and (NS1)13 were 12 and 15%, respectively. Each embryo line was initiated from an individual immature embryo explant derived from a single tree. This suggests that selecting for optimum maturation frequency amoung numerous embryogenic lines may be an important step toward developing an efficient regeneration system. The results in Table 2 also illustrate that modifying the development protocol is another way to improve maturation frequencies. The addition of BSO to the development medium doubled the maturation frequency in line (NS1)8. BSO is an inhibitor of reducing agents such as phenolics (Wann et al. 1986).

Germination The results of an experiment designed to test the effects of three culture treatments on somatic embryo germination are summarized in Table 3. Two treatments (slant and hanging) that avoided immersion of the radicle of somatic embryos in agar are compared to the control treatment where radicles were placed directly in the agar solidified medium. The mean frequency of somatic embryo germination was approximately doubled in the slant and hanging treatments over the control, and germination of the hanging treatment was significanlty higher than the control. Germination rates of somatic embryos were as high as 82% in both the slant and hanging treatments. More rapid root

growth occurred on the slant treatment than on the control or the hanging treatment.

Table 2. Maturation frequencies of Norway spruce somatic embryos.

Embryogenic callus line no.	Development protocol[1]	Somatic embryo	
		immature density[2]	maturation frequency
		(no./gr. callus)	(%)
(NS1)5	control	580	2.8
"	"	640	3.6
"	"	800	1.5
(NS1)13	control	150	16.0
(NS1)8	control	400 a	12.0 a
"	+ 10 µM BSO	330 a	25.0 b
"	+ 100 µM BSO	760 b	0.2 c

[1]Control development protocol as described in materials and methods.
[2]Mean of three counts on at least four replicate samples.

Table 3. Effect of three culture treatments on germination of Norway spruce somatic embryos.

Treatment[2]	N[3]	Somatic embryo[1]				Observation
		germination (%)		root length (mm)		
		mean	[range]	mean	[range]	
control	60	27a	[0-46]	2.3a	[0.5-4.0]	excessive radicle callus
slant	58	45ab	[20-82]	4.9b	[1.0-8.5]	most vigorous roots
hanging	58	56b	[25-82]	2.0a	[1.0-5.5]	most numerous root hairs

[1]Percentage of germination and primary root length measured at 21 days.
[2]Refer to materials and methods for description of treatments.
[3]N = number of somatic embryos per treatment.

Conversion A total of 31 somatic embryo plants were transferred to the greenhouse, and nine survived. The frequency of survival was higher when somatic plants were grown in soil in the growth chamber prior to transfer to the greenhouse (trial 1, refer to materials and methods), rather than direct transfer from in vitro to greenhouse conditions (trial 2). All nine of the somatic plantlets and six of the seven zygotic seedlings set a terminal bud during September. All somatic and zygotic plantlets survived overwintering.

When the plantlets were returned to 20 to 25°C, enlargement of the terminal buds occurred within 14 days on all somatic plantlets and six of the seven zygotic plantlets.

The growth flush of the terminal buds of the somatic plantlets occurred synchronously with the zygotic seedlings (Table 4 and Figure 2). Several plants began to flush on March 18 and within six days all somatic and six zygotic plants had flushed. The exception was one zygotic plantlet which had not set a resting bud until December (therefore it remained at warm temperatures for about 8 weeks longer than other plants) and did not obtain the same chilling period as the other somatic and zygotic plants. It flushed in mid-May.

Table 4. Initiation of vegetative growth flush from dormant terminal buds of somatic and zygotic plants.

Trial	Embryo origin	Number of plants overwintered	Number of plants initiating vegetative growth flush (3/18 to 3/24/87)	Mean plant height (cm) [range] (5/22/87)
1	somatic	3	3	9.7 a [6.5 - 15.5]
"	zygotic	7	6	13.3 a [8.0 - 19.0]
2	somatic	6	6	3.7 [1.5 - 6.0]

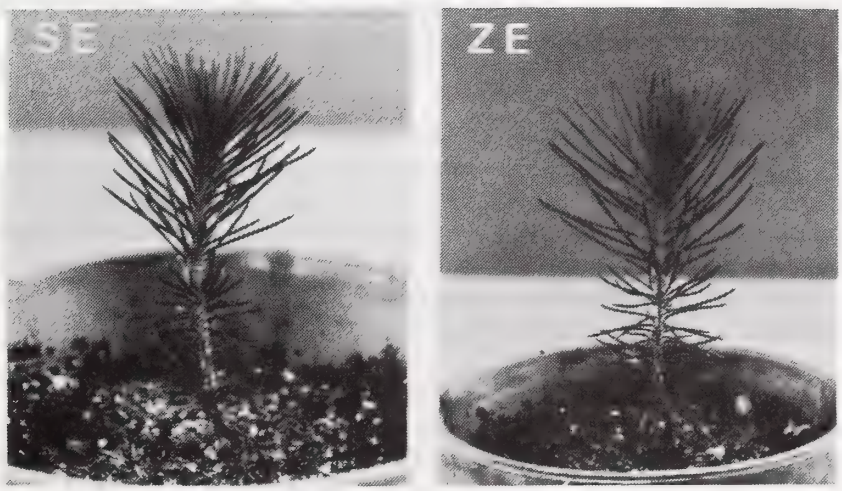


Figure 2. Initiation of secondary vegetative growth from somatic embryo plant (SE) synchronously with zygotic embryo plant (ZE). Photos taken 4/1/87, 14 days after bud break. Plant height, SE = 5.7 cm, ZE = 7.6 cm.

Statistically, there was no significant difference in the mean plant height between the somatic and zygotic plants in trial 1 (Table 4). Rigorous comparison of somatic and zygotic plants, however, is precluded for several reasons. The zygotic seedlings were not derived from the same tree as the somatic plants, and therefore difference could be genetic. Also, the sample size of three somatic plants in trial 1 is undesirably low. Our current efforts are aimed at producing large numbers of somatic plants via the optimum maturation, germination, and conversion techniques previously discussed, and at rigorous quantitative comparisons to zygotic plants derived from the same maternal tree.

In addition, direct comparisons between trial 1 and trial 2 plants are not valid. Trial 1 plants were chronologically older and 2-4 cm in height when transferred to the greenhouse, whereas trial 2 plants were about 1 cm in height when transferred and immediately set dormant terminal buds. Therefore, differences in plant height between trials are due to differences in experimental conditions and age.

CONCLUSIONS

We have utilized Norway spruce as a conifer model system for regeneration of plants via somatic embryogenesis. Our primary objective is to apply our results to conifers of commercial importance to the pulp and paper industry, namely, loblolly pine. Although we have initiated embryogenic callus in loblolly pine and several other commercially important conifers such as eastern white pine and white spruce (Wann et al. 1987, Nagmani et al. 1987, Becwar et al. 1987), we have not completed the regeneration process in these species. In the commercially important conifers the "bottle-neck" in regeneration appears to be the maturation process, similar to what we have reported here for Norway spruce. Therefore, understanding the biochemical basis for the reduced efficiency in spruce, and developing techniques for efficient conversion should be directly applicable to loblolly pine and other commercially important conifers.

Initiation from mature embryos of Norway spruce represents a first step toward utilizing mature explants, an aspect critical to development of "true-to-type" cloning in conifers. Taken collectively, our results on initiation from mature embryos and those reported by other workers (von Arnold and Hakman 1986) demonstrate that changes in and optimization of medium components can significantly effect initiation, and thus play a major role in extending the initiation window to more mature tissues.

The addition of BSO, an inhibitor of reducing agents, showed potential for significantly improving maturation frequency. Also the variation in efficiency among embryogenic callus lines derived from individual explants points out that there may be considerable genetic variation in maturation potential. Selecting for high maturation frequencies among callus lines coupled with refinements in the development protocol should result in considerable improvements.

Based on the highest mean efficiencies obtained for maturation (25%), germination (56%), and survival (conversion) of plants transferred to the greenhouse (29%), an overall efficiency of 4% was attained. Expressed relative to callus weight, about 8 grams of embryogenic callus of line (NS1)8 would be required to regenerate 100 plants. Although this efficiency is low and considerable improvement is needed for any commercial application, we are currently producing a sufficient number of somatic plants for detailed quantitative analysis of uniformity and growth characteristics.

Finally, an encouraging result in terms of the potential utility of embryogenesis in conifers is the complete regeneration of phenotypically normal plants from somatic embryos of Norway spruce. In terms of physiological response to changing environment, the somatic embryo plants have thus far responded (set dormant buds, overwintered, and initiated new vegetative growth) strikingly similar to control seedlings. To our knowledge this is the first demonstration of overwintering and initiation of new growth from dormant buds of conifer somatic embryo plants.

LITERATURE CITED

Amerson, H.V., Frampton, L.J., McKeand, S.E., Mott, R.L., and Weir, R.J. 1985. Loblolly pine tissue culture: laboratory, greenhouse, and field studies. In: Tissue Culture in Forestry and Agriculture. Eds., Henke, R.R., et al. Plenum Press, New York. pp. 271-287.

Becwar, M.R., Noland, T.L., and Wann, S.R. 1987a. Somatic embryo development and plant regeneration from embryogenic Norway spruce callus. Tappi J. 70(4):155-160.

Becwar, M.R., Noland, T.L., and Wann, S.R. 1987b. A method for quantification of the level of somatic embryogenesis among Norway spruce callus lines. Plant Cell Reports 6:35-38.

Becwar, M.R., Wann, S.R., and Kriebel, H.B. 1987. Initiation of embryogenic callus in Pinus strobus (eastern white pine) from immature embyro explants (Abstract). Genetic Manipulation of Woody Plants, Conf. June 21-25. Mich. St. Univ., East Lansing, MI.

Hakman, I. and Fowke, L.C. 1987. Somatic embryogenesis in Picea glauca (white spruce) and P. mariana (black spruce). Can. J. Bot. 65:656-659.

Hakman, I., Fowke, L.C., von Arnold, S., and Eriksson, T. 1985. The development of somatic embryos in tissue cultures initiated from immature embryos of Picea abies (Norway spruce). Plant Sci. 38:53-59.

Gupta, P.K. and Durzan, D.J. 1986. Somatic polyembryogenesis from callus of mature sugar pine embryos. Bio/Tech. 4:643-645.

Gupta, P.K. and Durzan, D.J. 1987. Biotechnology of somatic polyembryogenesis and plantlet regeneration in loblolly pine. Bio/Tech. 5:147-151.

Nagmani, R. and Bonga, J.M. 1985. Embryogenesis in subcultured callus of Larix decidua. Can. J. For. Res. 15:1088-1091.

Nagmani, R., Becwar, M.R., and Wann, S.R. 1987. Single-cell origin and development of somatic embryos in Picea abies (Norway spruce) and P. glauca (white spruce). Plant Cell Reports 6:157-159.

Redenbaugh, K., Paasch, B.D., Nichol, J.W., Kossler, M.E., Viss, P.R., and Walker, K.A. 1986. Somatic seeds: Encapsulation of asexual plant embryos. Bio/Tech. 4:797-801.

Smith, D.R., Singh, A.P., and Wilton, L. 1985. Zygotic embyros of Pinus radita in vivo and in vitro (Abstract). Int. Conifer Tissue Culture Work Group. Abstracts. Ed. Smith, D.R., New Zealand Forest Service, Rotorua, New Zealand.

von Arnold, S. and Eriksson, T. 1981. In vitro studies of adventitious shoot formation in Pinus contorta. Can. J. Bot. 59:870-874.

von Arnold, S. and Hakman, I. 1986. Effect of sucrose on initiation of embryogenic callus from mature zygotic embryos of Picea abies (Norway spruce). J. Plant Physiol. 122:261-265.

Wann, S.R., Feirer, R.P., Johnson, M.A., and Noland, T.L. 1986. Norway spruce as a model system for somatic embryogenesis in conifers. Proc. TAPPI Res. and Devel. Conf., Forest Biotech. Raleigh, NC, Sept. 28-Oct. 1, TAPPI Press, Atlanta, GA. pp. 131-135.

Wann, S.R., Johnson, M.A., Feirer, R.P., Becwar, M.R., and, Nagmani, R. 1987. Biochemical differences between embryogenic an nonembryogenic callus of Conifers (Abstract). Genetic Manipulation of Woody Plants, Conf. June 21-25. Mich. St. Univ., East Lansing, MI.

STAGE OF SHOOT DEVELOPMENT AND CONCENTRATION OF APPLIED HORMONE AFFECT ROOTING OF NORTHERN RED OAK SOFTWOOD CUTTINGS

R. M. Teclaw and J. G. Isebrands[1/]

Abstract.--Reliable techniques for vegetative propagation of northern red oak (NRO) are needed for physiological studies and tree-improvement programs because of the inherent variability of seedlings and the difficulty of rooting mature tissue. The objective of our study was to evaluate the effects of stage of shoot development and increased IBA concentration on rooting percent, number of roots, and root dry weights of softwood cuttings from NRO seedlings. Three concentrations of IBA (0.5%, 1.0%, and 1.5%) and blanching (light restriction with black rubber tubing), were evaluated for the above rooting variables on softwood cuttings taken from the terminal shoots of two- and three-flush NRO seedlings. The highest IBA concentration studied (1.5%) gave the highest rooting percent. Blanching at full leaf expansion, followed by hormone application 4 days later, produced the highest rooting in two-flush oaks. Similar trends were found in the number of roots and root dry-weight. However, rooting still declined with the age of the stock plant.

Additional keywords: Quercus rubra L., indole-butyric acid, vegetative propagation, blanching, Quercus morphological index (QMI), tree improvement.

Reliable techniques for vegetative propagation of northern red oak (Quercus rubra L.) are needed to achieve plant uniformity for morphological and physiological research, and for propagation of elite genotypes in tree improvement programs. Genetic variation inherent in open pollinated families limits the use of seedlings in physiological studies. Moreover, oak tree-improvement efforts have been limited because of the problems with collecting and storing acorns, the lengthy period needed for most oaks to reach seed-bearing age, and the difficulty in pollinating oaks (Irgens-Moller 1955, Beineke 1979). Clonal propagation of superior northern red oak (NRO) phenotypes would improve planting stock and produce genetically uniform material for research studies.

Oak is considered difficult to propagate and few investigators have been able to achieve commercial success of 70% or greater in rooting oak (Hartman and Kester 1983). In general, cuttings from young trees are more easily rooted than those from older trees (Farmer 1965, Duncan and Mathews 1969, Borzan et al. 1983). Probably the most significant improvements in rooting oak have come from advances in rooting hormones and in the

---

1/Biological laboratory technician and tree physiologist, respectively, USDA Forest Service, Forestry Sciences Laboratory, Rhinelander, WI 54501.

techniques used in their application. Cutting propagation for Quercus petraea and Q. robur have already been developed to an applicable scale in Germany (Kleinschmit 1986). In a previous study, Isebrands and Crow (1985) had little difficulty rooting one-flush NRO; but in subsequent trials rooting declined for second- and third-flush softwood cuttings. In these trials, cuttings of two- and three-flush oaks were all taken at approximately the same arbitrary morphological and physiological stages of shoot development. However, Spethmann (1982) has suggested that oak cuttings must be taken at a specific physiological stage in order to obtain rooting. Therefore, we investigated whether the stage of shoot development and concentration of applied hormone affects the rooting of softwood cuttings. This information is needed to develop a technique for vegetatively propagating mature superior NRO.

The results of three separate yet related experiments are reported here. The specific objectives were to determine if: A) age of cuttings (one-, two-, or three-flush) taken from NRO affects rooting; B) the concentration of an exogenous hormone affects rooting; and C) the stage of development within a flush affects rooting. The three experiments will be hereafter referred to as: Exp A - flush study, Exp B - hormone concentration study, and Exp C - stage-of-development study.

PROCEDURES

Acorns from a single open-pollinated NRO tree growing in Oneida County, Wisconsin were used for all three experiments. Seedlings were grown in 1:1 peat:sand mix, fertilized once a week with a modified Hoagland's solution, and watered as needed. Environmental conditions were set at 27-21°C day-night temperatures with a 16-hr photoperiod (Hanson et al. 1986).

In Exp A, the "best" hormone treatments from a previous rooting study (Isebrands and Crow 1985) were evaluated for rooting of softwood cuttings from one-, two-, and three-flush NRO seedlings. One of the following six treatments (Table 1, Exp A) was applied to 15 cm apical cuttings from plants at each of the three flushes. The treatments were: A1) a quick-dip application of 0.5% indole-butyric acid (IBA) in 95% ethanol (EtOH) preceded by blanching for 7 days, a technique that eliminates light to the rooting zone (the area of the stem where the IBA is applied and where the roots are to develop). A2) quick-dip application in 95% EtOH preceded by blanching; A3) no chemical application preceded by blanching; A4) 0.5% IBA in talc (with 15% benomyl[2/]) preceded by a 5 min soak in 1 $g.l^{-1}$ boric acid; A5) talc (with 15% benomyl) preceded by a 5 min soak in the boric acid solution, and A6) no application, i.e., control. When present, leaves were removed from the the rooting zone and the remaining leaves were cut back to about 1/3 their original size. Three replications of ten softwood

---

2/ Mention of trade names does not constitute an endorsement of the products by the USDA Forest Service.

cuttings for each of three flushes were treated; each cutting was inserted in a separate 350 ml pot with a 1:1 peat:sand mix, and then randomly placed on a mist bench with bottom heat at 27°C. Intermittent mist was applied at 5 sec bursts every 10 min in a glasshouse under 55% shade cloth. The percentage of rooted cuttings, the number of roots longer than 1 cm, and root dry-weight were determined for the various treatments after 60 days. Statistical analysis was with ANOVA for a complete randomized design. Rooting percentage data were transformed to arcsin $\sqrt{\%}$. Orthogonal contrasts were used to compare the treatments (Snedocor and Cochran 1967). Because of the difficulty in rooting NRO and the variability of the material, the 0.10 probability level was used to test contrasts.

Table 1.--Treatment applications for experiments A, B and C

Experiment A	Experiment B	Experiment C
Blanched	Blanched	Blanched
A1-0.5% IBA in EtOH	B1-0.5% IBA in EtOH	C1-0.8% IBA Pre-lag
A2-EtOH	B2-1.0% IBA in EtOH	C2-0.8% IBA Lag
A3-No chemical	B3-1.5% IBA in EtOH	C3-0.8% IBA Post-lag
Not Blanched	B4-No chemical	C4-95% EtOH Pre-lag
A4-0.5% IBA in talc	Not Blanched	C5-95% EtOH Lag
A5-talc	B5-0.5% IBA in EtOH	C6-95% EtOH Post-lag
A6-No chemical	B6-1.0% IBA in EtOH	
	B7-1.5% IBA in EtOH	
	B8-No chemical	

In Exp B, four concentrations of IBA (0%, 0.5%, 1.0%, and 1.5%, W:W) in 95% EtOH were applied as a quick-dip. Half the cuttings were blanched for 7 days and half were not blanched giving a total of eight treatments (Table 1, Exp B). Three replications of ten cuttings for each of the second and third flushes were treated. The cuttings were inserted in separate pots with 1:1 peat:sand mix and placed on a mist bench in a polyethylene greenhouse. The mist was controlled by a Mist-A-Matic misting device adjusted to keep the surface of the leaves wet throughout the 60-day rooting period. Rooting was analyzed as in Exp A.

In Exp C, two-flush NRO were grown to one of three developmental stages based on a Quercus morphological index (QMI) (Hanson et al. 1986). The seedlings were grown in a polyethylene greenhouse with environmental conditions, mix, containers, and nutrients similar to those described above.

When the developing leaves of the second flush attained a horizontal position, each seedling was assigned a specific stage of development (pre-lag, lag, or post-lag) for treatment (Table 1, Exp C). The lag stage as defined by Hanson et al. (1986) starts when the second leaf from the top of a flush is fully expanded. The seedlings were blanched 4 days before

they reached the specific stage and were then treated with either 95% EtOH or 0.8% IBA in 95% EtOH. Blanching times were estimated as closely as possible based on the development of the individual seedlings. Three replications of ten cuttings per treatment were used as in the other experiments. The cuttings were maintained throughout the rooting period, and the results analyzed as in Exp A.

RESULTS AND DISCUSSION

Experiment A

In the flush study, there were significant differences in rooting percentages among flushes and among treatments but no significant interaction between the flushes and treatments. All first-flush cuttings except the control rooted at 70% or higher (range 73-100%) (Table 2).

Table 2.--Flush Study (Exp A): Treatment means (± S.E.) for rooting variables of one-, two-, and three-flush NRO softwood cuttings.

Treatment	Rooting %	Number of roots	Root Dry Weight (mg)
1st FLUSH			
Blanched			
A1-0.5% IBA in EtOH	100 ± 0	14.4 ± 1.7	210 ± 41
A2-95% EtOH	90 ± 10	3.0 ± 0.1	79 ± 2
A3-No Chemical	83 ± 12	3.4 ± 0.7	124 ± 22
Not Blanched			
A4-0.5% IBA in Talc	93 ± 7	7.8 ± 0.7	140 ± 11
A5-Talc	73 ± 3	2.5 ± 0.5	44 ± 9
A6-No Chemical	53 ± 13	1.7 ± 0.4	51 ± 13
2nd FLUSH			
Blanched			
A1-0.5% IBA in EtOH	57 ± 23	2.2 ± 1.0	51 ± 22
A2-95% EtOH	70 ± 6	1.7 ± 0.6	152 ± 103
A3-No Chemical	27 ± 9	0.8 ± 0.3	16 ± 3
Not Blanched			
A4-0.5% IBA in Talc	20 ± 6	0.5 ± 0.3	8 ± 6
A5-Talc	10 ± 6	0.2 ± 0.1	4 ± 3
A6-No Chemical	13 ± 7	0.5 ± 0.3	6 ± 3
3rd FLUSH			
Blanched			
A1-0.5% IBA in EtOH	47 ± 9	1.6 ± 0.6	41 ± 10
A2-95% EtOH	13 ± 9	0.3 ± 0.3	2 ± 2
A3-No Chemical	7 ± 3	0.1 ± 0.0	6 ± 6
Not Blanched			
A4-0.5% IBA in Talc	3 ± 3	0.2 ± 0.2	2 ± 2
A5-Talc	3 ± 3	0.1 ± 0.1	1 ± 1
A6-No Chemical	3 ± 3	0.1 ± 0.0	1 ± 1
ALL THREE FLUSHES			
Blanched			
A1-0.5 IBA in EtOH	68 ± 11	6.1 ± 2.2	100 ± 30
A2-95% EtOH	58 ± 12	1.7 ± 0.4	78 ± 37
A3-No Chemical	39 ± 12	1.4 ± 0.6	49 ± 20
Not Blanched			
A4-0.5% IBA in Talc	39 ± 14	2.8 ± 1.3	50 ± 23
A5-Talc	29 ± 11	1.0 ± 0.4	16 ± 7.5
A6-No Chemical	23 ± 9	0.7 ± 0.3	20 ± 8.8

However, rooting percentage declined in the second and third flushes (range 10-57% and 3-47%, respectively). The highest rooting percentage for the first and third flushes and for the average of all three flushes was found with the blanched treatment and 0.5% IBA in EtOH. However, the quick-dip in 95% EtOH gave the highest rooting percentage for two-flush cuttings. Apparently, ethanol alone stimulates some root formation, but, as the age of the seedling increases, hormone application (IBA application) becomes increasingly important.

There were significant differences among flushes and treatments in the number of roots and the root dry-weights, and there were also significant interactions between flush and treatment. This suggests that treatment may vary with the age of the material. The marked differences of higher rootability in the more juvenile material (first-flush over second-flush over third-flush) was also evident in both the number of roots and root dry-weight. Even though all of our material was juvenile, but from different flushes, these results are consistent with previous reports that mature oaks are more difficult to root than juvenile material (Komissarov 1964, Farmer 1965, Isebrands and Crow 1985).

Blanching significantly increased rooting percentage, number of roots, and root dry-weight in all flushes (Table 3), and this improvement became more pronounced with cuttings from each subsequent flush. Rooting percentage was above 80% in all blanched treatments for the first flush, which again demonstrates the ease of rooting one-flush material. When IBA was applied in conjunction with blanching, the number of roots and root dry-weights were significantly higher for the first and third flushes than when no IBA was applied. These results indicate that light restriction can be used to improve rooting.

Table 3.--Flush Study (Exp A): Orthogonal linear contrasts for six treatments used to root softwood cuttings of northern red oak

	1st FLUSH Rooting			2nd FLUSH Rooting			3rd FLUSH Rooting		
Contrast	%	#	Dry Weight (mg)	%	#	Dry Weight (mg)	%	#	Dry Weight (mg)
Blanched vs. Not Blanched	**	**	**	**	**	**	**	**	**
Blanched: IBA vs. No IBA	NS	**	**	NS	NS	NS	**	**	**
Not blanched: IBA vs. No IBA	**	**	**	NS	NS	NS	NS	NS	NS
Blanched: EtOH vs. No EtOH	NS	NS	NS	**	NS	*	NS	NS	NS
Not Blanched: Talc vs. No Talc	NS	NS	NS	NS	NS	NS	NS	NS	NS

** = Significant at 0.05 probability level
* = Significant at the 0.10 probability level
NS = Not significant

Application of IBA with blanching did not significantly increase rooting percentage for the first flush because blanched cuttings without IBA also rooted well. This trend is supported by the findings of

Isebrands and Crow (1985) on the rooting of one-flush NRO. However, IBA does promote significantly more roots and higher root dry-weights, even in first-flush cuttings. There are no significant differences in rooting percentage, number of roots, and root dry-weights for blanched material of the second flush when comparing the IBA treatments versus treatments without IBA. Perhaps this is because ethanol by itself promoted rooting in juvenile material. However, all three rooting variables were significantly higher for IBA treatments versus no IBA treatment in the third flush, indicating the importance of hormone treatment for rooting this tissue. Talc alone had no significant effect.

Experiment B

In the IBA concentration experiment, there were significant differences between flushes and among treatments for rooting percentage and the number of roots. However, there were only significant differences among treatments and not between flushes for root dry-weight. Therefore, both flushes were pooled for analysis of root dry-weights. No significant interaction was found between flushes and treatments for all rooting variables. For blanched two-flush material the rooting percentage and number of roots increased with increasing IBA concentration to 83% rooting and 3.1 roots per cutting for 1.5% IBA (Table 4). Unblanched material generally rooted at a lower rate than blanched. This result reinforces the importance of blanching in conjunction with hormone application. Rooting decreased more in the third flush than in the second flush and increased with IBA concentration. The values for the rooting variables for the blanched 1.0% IBA treatment of the third flush appear to be unexplainably low and are not consistent with trends. These low values could mask the statistical significance of some treatments. The greatest root dry-weight was for blanched material treated with 1.5% IBA, although there were no significant differences among concentrations of IBA in both blanched and unblanched material.

Table 4.--Hormone concentration study (Exp B): Treatment means (± S.E.) for rooting variables of two- and three-flush NRO cuttings.

Treatment	2nd Flush		3rd Flush		Pooled 2nd & 3rd Flush
	% Rooting	# Roots	% Rooting	# Roots	Root Dry Weight (mg)
Blanched					
B1-0.5% IBA	60 ± 12	1.8 ± 0.7	50 ± 8	1.0 ± 0.2	125 ± 47
B2-1.0% IBA	77 ± 3	2.8 ± 1.1	33 ± 3	0.7 ± 0.2	80 ± 27
B3-1.5% IBA	83 ± 12	3.1 ± 0.1	60 ± 8	2.0 ± 0.4	130 ± 19
B4-No Chemical	43 ± 9	1.1 ± 0.4	23 ± 3	0.4 ± 0.1	25 ± 8
Not Blanched					
B5-0.5% IBA	67 ± 3	1.7 ± 0.3	43 ± 9	1.0 ± 0.2	64 ± 11
B6-1.0% IBA	57 ± 18	1.8 ± 0.5	57 ± 7	1.5 ± 0.7	108 ± 31
B7-1.5% IBA	57 ± 13	1.6 ± 0.4	63 ± 7	1.9 ± 0.4	82 ± 15
B8-No Chemical	30 ± 17	0.7 ± 0.5	23 ± 9	0.5 ± 0.2	22 ± 10

Blanching alone significantly increased the rooting of two-flush but not three-flush material. This phenomenon is probably a result of low rooting values for the 1.0% IBA blanched treatment of the third flush. Application of IBA in conjunction with blanching significantly increased the rooting for both flushes. The application of IBA on unblanched material significantly increased rooting for both flushes except for the number of roots of two-flush cuttings. However, the number of roots was significant at the 12% probability level. These results illustrate the importance of IBA application in rooting of NRO softwood cuttings. Chong (1980) rooted English oak (Quercus robur 'Fastigiata') with 2.0% IBA. Increasing the IBA concentration above 1.5% may increase rooting of the more mature NRO material.

Experiment C

IBA application significantly increased rooting for all developmental stages. This result is clearly illustrated by the means of the rooting percentages. Rooting with IBA treatments ranged from 60 to 70% and without IBA ranged from 33 to 53% (Table 5). Although the differences in rooting percentage for pre-lag and post-lag cuttings (60 and 70%, respectively) were not significantly different, the post-lag treatment had significantly greater root dry-weights. This finding again shows the importance of IBA application to rooting. Although the IBA did not significantly increase rooting percentage, it did significantly increase the mass of the root system. The insignificant differences can probably be attributed to our small sample size (only 30 individuals per treatment) and to the high standard errors. In general, treating the cutting with IBA at a later stage of development apparently is the "best" method for rooting NRO softwood cuttings.

Table 5.--Stage-of-development study (Exp C): Treatment means (± S.E.) for rooting variables of different aged cuttings.

	Treatment	Rooting %	# of Roots	Root Dry Weight (mg)
C1	0.8% IBA - Pre-lag	60 ± 10	2.0 ± 0.0	113 ± 20
C2	0.8% IBA - Lag	63 ± 9	1.9 ± 0.3	100 ± 19
C3	0.8% IBA - Post-lag	70 ± 12	2.7 ± 0.9	256 ± 87
C4	Control Pre-lag	33 ± 7	1.3 ± 0.6	61 ± 26
C5	Control Lag	53 ± 9	1.0 ± 0.3	117 ± 41
C6	Control Post-lag	53 ± 13	1.4 ± 0.5	170 ± 49

CONCLUSIONS

Juvenile one- and two-flush NRO cuttings can be easily rooted at or above commercially acceptable levels using established rooting techniques. This conclusion is based on consistent results obtained on two-flush material in all three experiments. However, as the material matures, rooting becomes more difficult and new techniques need to be developed. Increased IBA concentrations, possibly as high as 2.0%, and aryl esters of

IBA have proven effective with other species (Haissig 1983, Struve and Arnold 1986) and need to be tested on NRO. In addition, blanching significantly improved rooting. Vegetative propagation of superior northern red oak phenotypes will require further advances in rooting techniques.

LITERATURE CITED

Beineke, W. 1979. Tree improvement in the oaks. Proc. Regenerating Oak in Upland Hardwood Forests. Purdue U., Lafayette, Indiana, Feb. 22-23, 1979. p. 126-132.

Borzan, A., A. Krstinic, W. Libby and M. Vidakovic. 1983. The rooting of cuttings of the early and late flushing Slavonian oak. Ann. pro. exp. foresticis 21: 213-222.

Chong, C. 1980. Influence of high IBA concentrations of rooting. Proc. Int. Plant Prop. Soc. 30: 453-460.

Duncan, H. and F. Mathews. 1969. Propagation of southern red oak and water oak by rooted cuttings. USDA For. Service. Res. Note SE-107.

Farmer, R. E., Jr. 1965. Mist propagation of juvenile cherrybark oak by cuttings. J. For. 63: 463-464.

Haissig, B. E. 1983. N phenyl indole-3-butyramide and phenyl-3-chiolohydrate enhance adventitious root primordium development. Physiol. Plant. 57: 435-440.

Hanson, P. J., R. E. Dickson, J. G. Isebrands, T. R. Crow, and R. K. Dixon. 1986. A morphological index of Quercus seedlings ontogeny for use in studies of physiology and growth. Tree Phys. 2: 273-281.

Hartmann, H. and D. Kester. 1983. Plant propagation. Prentice-Hall. Englewood Cliffs, NJ. 727 pp.

Irgens-Moller, H. 1955. Forest tree genetics research: Quercus L. Econ. Bot. 9: 53-71.

Isebrands, J. G. and T. R. Crow. 1985. Techniques for rooting juvenile softwood cuttings of northern red oak. Proc. 5th Central Hardwood forest conference. Univ. of Ill., Urbana-Champaign, Ill. pp. 228-223.

Kleinschmit, J. 1986. Oak breeding in Germany-Experiences and problems. Proc. IUFRO joint meeting of working parties breeding theory, progeny testing and seed orchards. Williamsburg, Virginia. Oct. 12-17, 1986 (In press).

Komissarov, D. A. 1964. Biological basis for propagation of woody plants by cuttings. Trans. Israel Program Sci. Translations 1968. TT68-50372.

Snedocor, G. and W. Cochran. 1967. Statistical methods. Iowa State University Press. Ames, IA. 193 pp.

Spethmann, W. 1982. Cutting propagation of deciduous trees. I. Experiments with maple, ash, oak, beech, cherry, lime tree and birch. Allg. Forst. u J. Ztg. 153(1-2) 13-23.

Struve, D. K. and M. A. Arnold. 1986. Aryl esters of IBA increase rooted cutting quality of red maple 'Red Sunset' softwood cuttings. Hort. Science 21(6): 1392-1393.

BIOTECHNOLOGY II

MODERATED BY DR. R. J. DINUS

Institute of Paper Chemistry

OSMOTIC ADJUSTMENT IN PINUS TAEDA FAMILIES IN RESPONSE TO WATER STRESS

C.E. Meier[1/] and R.J. Newton[2/]

Abstract--Osmotic adjustment is often critical for seedling survival under drought stress. This study examined: (1) the potential of cyclic soil water deficits to induce osmotic adjustment in Pinus taeda L. seedlings from two half-sib families, the one from a xeric provenance, the other a mesic, and (2) the influence of these water deficits on cell wall elasticity and symplasmic volume. Seedlings were grown for five months under low stress conditions in an environmental chamber. Water deficits were induced by withholding soil water. Predawn xlyem water potentials did not exceed -0.6 MPa in the daily watered control treatment while in the stress treatment, seedlings were watered to saturation only when predawn xylem potentials reached -1.5 MPa (once/12-13 days). After four stress cycles, psychrometric analysis indicated a significant osmotic adjustment of -0.42 MPa relative to the controls. There was no significant difference in adjustment between families. Pressure-volume curve analysis confirmed significant adjustment and indicated that cell wall elasticity and symplasmic volume declined significantly due to stress. Results suggest osmotic adjustment is a common mechanism of drought resistance in Pinus taeda.

Additional keywords: loblolly pine, cell wall elasticity, pressure-volume, symplasmic volume

INTRODUCTION

The maintenance of cell tugor as soil moisture and/or plant water potential (Ψ) declines is critical for normal cell function and plant survival. Stomatal closure, photosynthesis, cell expansive growth and cell division are highly sensitive to turgor potential (Ψ_p) (Turner and Jones 1980, Bradford and Hsiao 1982). A number of physiological mechanisms interact to maintain Ψ_p. Among them osmotic adjustment, because of its fundamental position as a water potential component, has received the most attention (e.g. Hinckley et al. 1980, Meinzer et al. 1986, Sobrado 1986, Joly and Zaerr 1987). Osmotic adjustment is a decrease in the symplastic osmotic potential (Ψ_π) (Parker and Pallardy 1985); it results from an increase in solute concentration within the symplasm. The adjustment can be caused by an active net accumulation of solutes in the symplasm or alternatively, a passive increase in solute concentration due to tissue dehydration or change in partitioning of water between the symplasm and apoplasm (Joly and Zaerr 1987). A decline in $\Psi\pi$ caused by the first mechanism is true adjustment (Hinckley et al. 1980).

1/2/ Assistant and Associate Professors, repectively; Department of Forest Science and the Texas Agricultural Experiment Station, Texas A&M University System, College Station, Texas. This research was supported by the Texas Agricultural Experiment Station ERA program.

While osmotic adjustment is a normal process in many woody plants (Bowman and Roberts 1985, Meinzer et al. 1986, Sobrado 1986) it has attracted recent attention because of the need to increase early drought resistance of outplanted tree seedlings. If seedling osmotic adjustment can be enhanced by sublethal preconditioning with drought stress then survival may be increased (Parker and Pallardy 1985, Seiler and Johnson 1985, Joly and Zaerr 1987).

Pinus taeda is the most important commercial pine species in Texas and the southeastern United States. In Texas and the Western Gulf drought stress is the single greatest cause of seedling mortality (Karr et al. 1984). It has been demonstrated that in Pinus taeda, preconditioning drought stress can induce at least apparent osmotic adjustment (Hennessey and Dougherty 1984, Seiler and Johnson 1985). Yet, preliminary work has indicated that rapidly induced severe drought stress may result in minimal osmotic adjustment (Wilson 1985) and there may be major differences between families of Pinus taeda in ability to osmotically adjust (Holmes 1986).

The objective of this study was to evaluate the potential for inducing osmotic adjustment by cyclical slow development of high sublethal water deficits and to examine the ability of contrasting Pinus taeda families, to osmotically adjust.

METHODS

Plant materials and growth conditions

Seedlings were grown from seed collected in seed orchards from two half-sib families. The one family (GR1-8), provided by the Texas Forest Service, originated from the western edge of the species natural range and is known for superior early survival and growth on xeric sites. The other (8-76), provided by Weyerhaeuser Co. originated from the North Carolina Coastal Plain and is known for its superior early growth on mesic sites.

Plants were grown in one liter - Rootainers ; (Spencer-Lemaire Industries, Ltd.) filled with fritted-clay. This media was selected because it retains large amounts of water relatively uniformly absorbed over the -0.1 to -1.5 MPa range of soil water potentials and has excellent aeration characteristics (van Bavel et al. 1978). Seedlings were watered daily. For five days of each week they received 85 ml of a fertilizer solution developed for pine seedlings (Miller 1982). Then, for the remaining two days all containers were watered to saturation with distilled water to limit salt build-up.

Seedlings were grown in a controlled environment chamber during the entire experiment. Temperature and relative humidity were maintained at 25 $\pm$ 1 °C and 87 $\pm$ 5%, respectively. Day length was 16 hours with 640 μE m^{-2} sec^{-2} of photosynthetically active radiation at plant height. Seedlings were rotated within the chamber to assure uniform growth environment.

Drought Stress Treatment and Measurement Methods

After five months seedlings were randomly assigned to control and drought stress treatments. All fertilization was stopped. The control treatment received 90 ml of distilled water daily. In the stress treatment water was

withheld until predawn water potentials reached ≈ -1.5 MPa. Then all seedlings were watered to saturation for two days. Predawn fascicle water potentials were determined daily for all treatment - seed source combinations using the pressure chamber technique (Ritchie and Hinckley 1975) with the aid of a dissecting microscope (40x) to detect pressure balance points. After each drought stress cycle, seedlings from each treatment seed source combination were randomly selected for analysis of osmotic adjustments (Seiler and Johnson 1985). In the first two cycles five seedlings per family-treatment combination were analyzed. This number was increased to 10 in later cycles.

The root systems of selected seedlings were immersed in distilled water and seedling shoots were covered with plastic bags. Contact between the foliage and bag was minimized. Seedlings were then allowed to rehydrate at 3 ± .5°C for 36 to 48 hours. After rehydration the water potentials (Ψ) of two fascicles per seedling were determined by use of previously described pressure chamber methodology. Simultaneously, two mature needles were taken from each seedling for measurement of osmotic potential. Needles were frozen in liquid nitrogen and osmotic potential was determined psychrometrically (Wescor, Inc., models HR33T, C-52, Logan UT) using the expressed sap method of Turner et al. (1978). Measurements were not corrected for non-symplasmic water. Turgor potentials were calculated as: $\Psi_p = \Psi - \Psi_\pi$.

After apparent osmotic adjustment had been detected by the above technique seedlings were subjected to an additonal treatment cycle. Then bulk shoot water relations were characterized from pressure-volume curves (Tyree and Hammel 1972) for shoots hydrated as previously described. Osmotic potentials at full saturation ($\Psi_{\pi o}$) and the turgor loss point ($\Psi_{\pi p}$), symplastic water fraction (SWF) and relative water content at the turgor loss point (RWC_p) were estimated using methods described by Cheung et al. (1975). Maximum bulk modulus of elasticity (ξ_{max}) was derived following the method of Bowman and Roberts (1985). Ten seedlings per family-treatment combination were analyzed.

RESULTS

In the stress treatment predawn xylem water potentials were higher (Figure 1) in the GR 1-8 family and declined at a slower rate than in the 8-76 family. This difference was primarily due to the smaller size and thus smaller transpiring surface of the GR1-8 family (Table 1). There was no significant difference between families in mid-day stomatal resistance (unpublished data), which might also have been a factor. As shown in Table 1 the 8-76 family showed a more rapid growth rate before treatment and, even under stress, nearly doubled in shoot mass during the stress treatment period. The GR1-8 family exhibited a consistently more conservative growth rate.

After four drought stress cycles significant differences in water potential components were observed between families and treatments (Table 2). Rehydration of seedlings prior to measurement increased xylem water potentials, but stressed seedlings still had significantly, slightly lower (-0.10 MPa) Ψ values (Table 2). In both families Ψ_π values were also significantly influenced by the cyclic moisture stress treatment, averaging a near identical -0.43 MPa lower in the rehydrated stressed seedlings. Because of the decreased Ψ_π values in the seedlings preconditioned by cyclic stress, Ψ_p values were significantly higher than in the control treatments; the two families again averaged a near identical, significant increase of 0.34 MPa due to treatment.

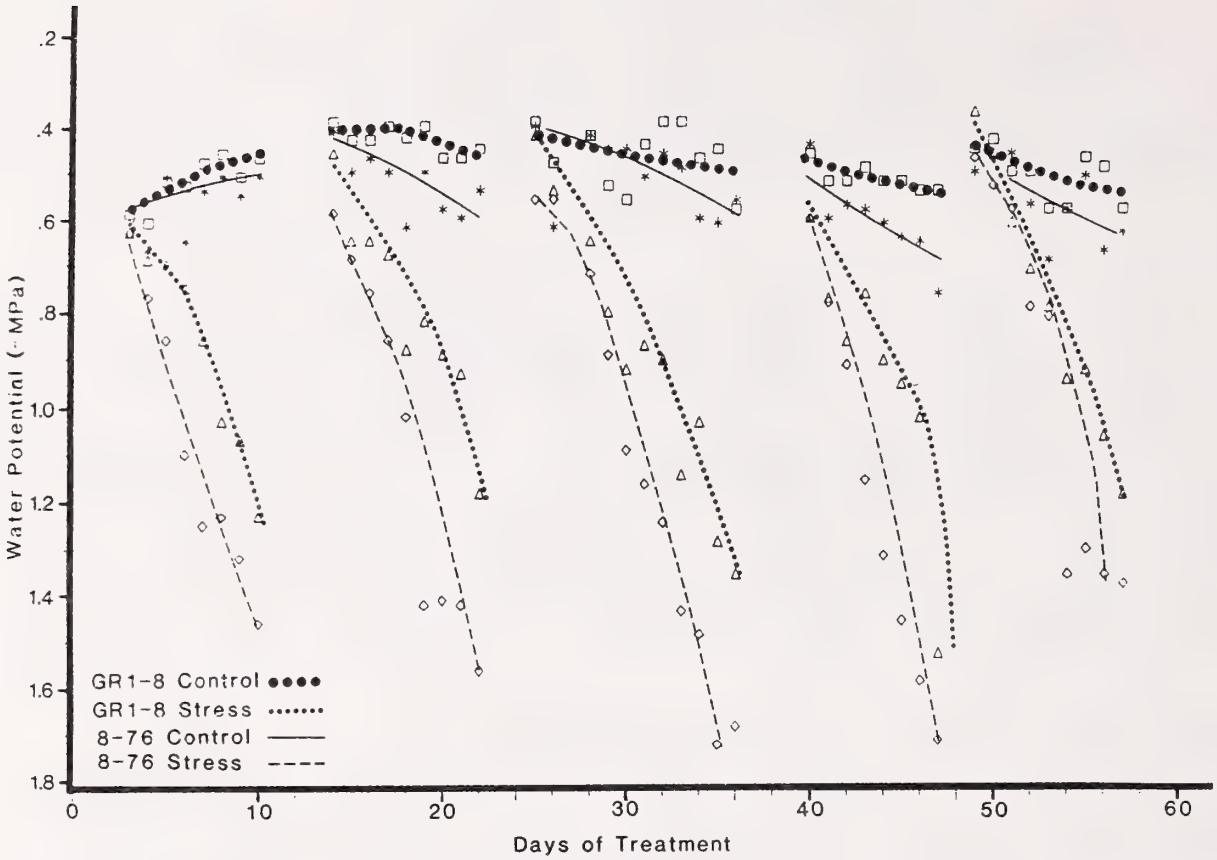


Figure 1. Predawn needle water potentials of loblolly pine seedlings from two families (GR1-8, 8-76) under control (daily watered) and stress (water withheld) treatments. All treatments watered to saturation when stress treatment reached approximately ≈ -1.5 MPa.

All Ψ, Ψ_π, and Ψ_p values for family 8-76 differed significantly from their treatment counterparts in GR1-8 (Table 2). Differences between families in Ψ while statistically significant, were minimal, averaging -0.09 MPa lower in the 8-76 family. Osmotic and turgor potentials differed by an average of -0.21 and 0.12 MPa between families with the 8-76 having the lower and higher value, respectively. It should be noted that pattern of difference in Ψ_p relative to Ψ and Ψ_π was predictable because of the way Ψ_p was estimated.

To provide confirmation and to elucidate the above trends, pressure-volume curve analyses were performed. In both families, osmotic potentials at full saturation were significantly lower in the seedlings preconditioned by cyclic stress (Table 3). However, $\Psi_{\pi o}$ values for control and stressed seedlings from the 8-76 family were significantly lower than those of counterpart seedlings in the GR1-8 family by -0.1 and -0.24 MPa, respectively. Comparison of $\Psi_{\pi o}$ values (Table 3) with Ψ_π values near saturation (Table 2) indicates that psychrometrically determined values, are as expected lower and differences between treatments more pronounced in the latter measurements. These differences reflect differences in degree of rehydration between treatments and families and analytical methods. The latter is supported by practical and theoretical considerations (Ritchie and Dunham 1979) and the former by observed

variance between treatments and families in $\Psi_{\pi o}$ (Table 3). However, the concurrence of pattern in significant differences between families and treatments supports the validity of osmotic adjustment estimated psychrometrically (Table 2).

Table 1. Comparison of Seedling growth characteristics of two Pinus taeda families under drought stress treatments.

Family	Height (cm)	Root Collar Diameter (mm)	Shoot Dry Mass (gm)	Root Dry Mass (gm)
		Pretreatment*		
8-76	23.1[a]**	5.9[a]	6.5[a]	2.2[a]
GR1-8	16.5[b]	4.9[b]	4.4[b]	1.1[b]

	Post treatment							
	Control	Stress	Control	Stress	Control	Stress	Contol	Stress
8-76	25.4[a]	28.2[a]	8.4[a]	8.1[a]	14.3[a]	12.0[a]	5.9[a]	4.1[a]
GR1-8	18.1[b]	18.2[b]	6.2[b]	5.8[b]	6.9[b]	7.1[b]	2.1[b]	1.9[b]
Treatment	N.S.***		N.S.		N.S.		S.	
Family	S.		S.		S.		S.	
Trt x Family	N.S.		N.S.		N.S.		S.	

* All treatment-family combinations increased significantly ($P<.05$) during treatment.

** Statistically significant differences ($P<.05$) between families within measurement periods are noted by different superscript letters.

***S. and N.S. denotes significance and nonsignificance, respectively at the 0.05 level.

Table 2. Needle water (Ψ), osmotic (Ψ_π), and turgor (Ψ_p) potentials in Pinus taeda families as influenced by cyclic drought stress.

Family	Treatment	Ψ	Ψ_π	Ψ_p
		(MPa)	(MPa)	(MPa)
8-76	control	-.41	-1.30	.89
	stress	-.50	-1.72	1.22
GR1-8	control	-.31	-1.08	.75
	stress	-.41	-1.52	1.11
Treatment:		S.*	S.	S.
Family:		S.	S.	S.
Trt x Family:		N.S.	N.S.	N.S.

*S. and N.S. denote significance and non-significance, respectively, at the 0.05 level.

Table 3. Comparison of osmotic potential at full saturation ($\Psi_{\pi o}$), osmotic potential at turgor loss point ($\Psi_{\pi p}$), bulk molulus of elasticity (ξ_{max}), relative water content at turgor loss point (RWC_p) and symplasmic water fraction (SWF) between drought stress levels and two families of Pinus taeda.

Family	Treatment	$\Psi_{\pi o}$	$\Psi_{\pi p}$	ξ_{max}	RWC_p	SWF
		(MPa)	(MPa)	(MPa)		
8-76	control	-1.38	-1.95	10.6	.90	.36
	stress	-1.60	-2.18	17.3	.92	.32
GR1-8	control	-1.28	-1.89	12.0	.89	.36
	stress	-1.40	-2.09	21.2	.91	.27
Treatment:		S.*	S.	S.	S.	S.
Family:		S.	N.S.	N.S.	N.S.	N.S.
Trt x Family:		N.S.	N.S.	N.S.	N.S.	N.S.

*S. and N.S. denote significance and non-significance, respectively, at the 0.05 level except for SWF which is at the 0.08 level.

In the other water relations parameters measured (Table 3) there was not a significant difference between families. However, within families cell wall elasticity decreased (higher ξ_{max}) significantly due to the stress treatment; RWCp showed a slight but significant increase in the seedlings subjected to cyclic stress; $\Psi_{\pi p}$ declined in seedlings under stress; and the decline in SWF under stress was significant at the 0.08 level.

DISCUSSION

Evaluation of tissue water relations by both psychrometric and pressure-volume methods have indicated significant osmotic adjustment, due to water stress. The appoximately 0.4 MPa decrease in Ψ_{π} of stressed seedlings, (Table 2), would allow for maintenance of turgor to needle water potentials approximately 0.4 MPa lower then controls. Stressed seedlings had 0.10 MPa lower Ψ values with Ψ_p values approximately 0.34 MPa higher than control seedlings. These magnitudes are similar to those reported by Seiler and Johnson (1985) for a single Pinus taeda source. The $\Psi_{\pi o}$ values (Table 3) are similar in magnitude to those reported by Hennessey and Dougherty (1984), and magnitudes of adjustment, especially of the 8-76 family, agree well with their results.

In comparing levels of stress, it should be noted that the cyclic stress which Seiler and Johnson (1985) imposed approximated the level in this study; however, the cyclic stress which Hennessey and Dougherty (1984) imposed had a minimum value of -7.5 MPa. Thus, their stress treatment was roughly equivalent to our control treatment in minimum xylem water potential. For this reason, magnitudes of osmotic adjustment reported here may be underestimates of the total osmotic adjustments which stressed seedlings undergo relative to minimally stressed individuals.

Osmotic adjustment in the stressed seedlings appears due to both active and passive process. Declines in $\Psi_{\pi o}$ of stressed seedlings can be partially

explained by decreases in SWF, a passive adjustment process; however, the magnitudes of osmotic adjustment (Table 2, 3) strongly suggest an active process is also present.

Decreases in SWF are often associated with production of cells of decreasing size and increased cell wall thickness by plants under drought stress. This explanation is in agreement with the significant growth which occurred during treatment and supported by the higher ξ_{max} and RWC_p in stressed seedlings, both of which are indicative of decreased cell wall elasticity (Parker and Pallardy 1985).

In summary there is a significant decrease in $\Psi_{\pi o}$ and $\Psi_{\pi p}$ for the preconditioned seedlings. Thus, osmotic adjustment has occurred. While it appears that both active and passive processes are interacting in the development of the observed osmotic adjustment the presence of true osmotic asjustment appears evident. This combination of factors, a high ξ_{max} and a low Ψ_{π} (large osmotic adjustment) as pointed out by Bowman and Roberts (1985) is not in conflict. Together they can create large relatively rapid decreases in symplasmic water potential which facilitates continued water uptake while maintaining positive turgor and cell expansive growth.

Literature Cited

Bowman, W.D. and S.W. Roberts 1985. Seasonal changes in tissue elasticity in chaparral shrubs. Physiologia Plantarum 65:233-236.

Bradford, K.J. and Hsiao, T.C. (1982). Physiological responses to moderate water stress. pp.263-324. In Lange, O.L., P.S. Nobel, C.B. Osmond and H. Ziegler (eds.). Encylopedia of Plant Physiology, Vol 12B Physiolobical Plant Ecology. Springer-Verlog, Berlin.

Cheung, Y.N.S., M.T. Tyree, and J. Dainty 1975. Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. Canadian Journal of Botany 53:1342-1346.

Hennessey, T.C. and P.M. Dougherty 1984. Characterization of the internal water relation of loblolly pine seedlings in response to nursery cultural treatments: implications for reforestation success. pp. 225-243 in Durgea, M.L. and G.N. Brown (eds.) Seedling Physiology and Reforestation Success. Martinus Nijhoff/ Dr. W. Junk Publishers, Boston, pp 326.

Hinckley, T.M., F. Duhme, A.R. Hinckley, and H. Richter 1980. Water relations of drought-hardy shrubs: osmotic potential and stomatal reactivity. Plant, Cell and Environment 3:131-140.

Holmes, P.D. 1986. Response of drought and nutrient stressed loblolly pine grown in native and overburden material from the post oak-savannah of Texas. Doctoral Dissertation, Texas A&M University, College Station, Texas, 180pp.

Joly, R.J. and J.B. Zaerr 1987. Alteration of cell-wall water content and elasticity in Douglas-fir during periods of water deficit. Plant Physiology 83:418-422.

Karr, B.L., J.B. Baker, T. Monagham (eds.) 1984. Proceedings of the Symposium on The Loblolly Pine Ecosystem (West Region). Mississippi cooperative Exteneion Service, Extension Forestry Department, Mississippi State, Mississippi, 340 pp.

Meinzer, F.C., P.W. Randel, M.R. Sharifi and E.T. Nilsen 1986. Turgor and osmotic relations of the desert shrub Larrea tridentata. Plant, Cell and Environment 9:467-475.

Miller, D.E. 1982. Early Selection of Loblolly Pine Based on Genotype x Fertilizer Interaction of Seedlings. Master of Science Thesis, Texas A&M University, College Station, Texas, 91pp.

Parker, W.C. and S.G. Pallardy 1985. Genotypic variation im tissue water relations of leaves and roots of black walnut (Juglans nigra) seedlings. Physiologia Plantarum 64:105-110.

Ritchie, G.A. and T. M. Hinkley 1975. The pressure chamber as an instrument for ecological research. Advances in Ecological Research 9:165-254.

Ritchie, G.A. and D.L. Dunham 1979. Pressure-volume curves I. theory, methods and interpretation. Weyerhaeuser Forest Research Technical Report 042-2203/79, 16pp.

Seiler, J.R. and J.D. Johnson 1985. Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. Forest Science 31:742-749.

Sobrado, M.A. 1986. Aspects of tissue water relations and seasonal changes of leaf water potential components of evergreen and deciduous species coexisting in tropical dry forests. Oecologia 68:413-416.

Turner, N.C., J.E. Begg, and M.L. Tonnet 1978. Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at whict stomata close. Australian Journal of Plant Physiology 5:597-608.

Turner, N.C. and M.M. Jones 1980. Turgor maintenance by osmotic adjustment: a review and evaluation. pp 87-103 In N.C. Turner and P.J. Kramer (eds.) Adaptation of Plants to Water and High Temperature Stress. Wiley-Interscience, New York.

Tyree, M.T. and H.T. Hammel 1972. The measurement of turgor pressure and the water relations of plants by the pressure-bomb technique. Journal of Experimental Botany 23:267-282.

Van Bavel, C.H.M., R. Lascano, and D.R. Wilson 1978. Water relations of fritted clay. Soil Science Society of America Journal 42:657-659.

Wilson, A.B. 1985. The influence of Phosphorous Nutrition and Water Stress on the Osmotic Adjustment and Growth of Loblolly Pine. Master of Science Thesis, Texas A&M University, College Station, Texas, 71pp.

GROWTH AND ISOZYME ANALYSIS OF AXENIC CULTURES OF *CRONARTIUM QUERCUUM* F. SP. *FUSIFORME* DERIVED FROM BASIDIOSPORES

S. F. Shamoun[1/], F. H. Huang[2/] and D. A. Slack[3/]

Abstract.--Fusiform rust, induced by the pathogen *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (*c.q.f.*), is a heteroecious macrocyclic rust fungus and the most economically destructive disease on pine plantations in the Southeastern U.S.

One of the primary advantages of axenic hyphal cultures is to study the physiology of rust resistance. Also, biochemical comparisons of the mycelium and the uninfected Loblolly pine host may indicate whether certain chemical resistance (isozymes) found in infected tissues of resistant Loblolly pine families are of fungus (*c.q.f.*) or host origin. The isozyme markers in tree improvement breeding programs can save researchers time over present conventional methods of inoculating pine and waiting for 6-9 months for gall formation.

1/ Graduate Assistant, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR.

2/ Associate Professor, Dept. of Horticulture and Forestry, University of Arkansas, Fayetteville, AR.

3/ Professor, Dept. of Plant Pathology, University of Arkansas, Feyetteville, AR.

CHLOROPLAST DNA VARIATION IN THE SYMPATRIC ZONE OF LODGEPOLE PINE (*PINUS CONTORTA* DOUGL.), AND JACK PINE (*P. BANKSIANA* LAMB.).

D. B. Wagner,[1] D. R. Govindaraju[2] and B. P. Dancik [3]

A chloroplast DNA restriction fragment length polymorphism (RFLP) and its relationship with cone morphology was studied in a sympatric population of lodgepole pine and jack pine. Novel cpDNA variants, found only in or near the sympatric region, occurred in approximately 5% of the individuals of this population. Chloroplast DNA typical of jack pine was associated with cone morphology typical of lodgepole pine and hybrids, suggesting that cpDNA of jack pine may be functional in the nuclear genetic background of lodgepole pine.

Additional keywords: Restriction fragment length polymorphism (RFLP), cpDNA polymorphism, sympatric zone.

An understanding of the levels of genetic variation in natural populations is fundamental to breeding programs. Patterns of genetic variation in forest trees traditionally have been described on the basis of provenance studies (Zobel and Talbert 1984), and also using biochemical genetic markers such as allozymes or monoterpenes (Ledig 1986, Kinloch et al. 1986). Recently, the availability of molecular technology has stimulated interest in studying the amount and distribution of genetic variation in forest trees at the molecular level (Neale et al. 1986, Wagner et al. 1987).

Genetic variation occurs in both nuclear and extranuclear (chloroplast and mitochondrial) genomes. It is important to understand extranuclear genetic variation because 1) it is yet unclear whether this variation has phenotypic implications in forest trees, 2) this variation may provide uniparentally inherited markers useful in monitoring pedigrees, and 3) if extranuclear variation is to be incorporated into tree improvement programs by traditional or biotechnological approaches, its interaction with nuclear variation must be understood.

The chloroplast genome has several interesting features: a) it is a relatively small circular molecule whose size varies from 120 kb to 180 kb (Palmer and Thompson 1982); b) a number of important genes, such as those responsible for RUBP carboxylase and herbicide resistance, have been mapped to the molecule; c) although it is considered to be evolutionarily conserved, portions of it appear to be highly polymorphic; and d) chloroplasts are

---

[1] Assistant Professor, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[2] Research Associate, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[3] Professor, Department of Forest Science, University of Alberta, Edmonton, Alberta. This work was supported in part by a USDA grant (85-FSTY-9-0149 to D.B.W.

maternally inherited in the majority of plants, but their inheritance in conifers may be paternally biased (Neale et al. 1986, Wagner et al. 1987). Paternal inheritance provides diagnostic markers for evolutionary and breeding studies, that may be unique to conifers.

We report here chloroplast DNA variation and its statistical relationship with cone morphology in a sympatric population formed by jack and lodgepole pines (figure 1). These two species originated from an ancestral complex and diverged into the present day species in the late Tertiary (Critchfield 1957, Yeatman 1967). The formerly geographically separated taxa have recently become sympatric in western Canada (Moss 1949).

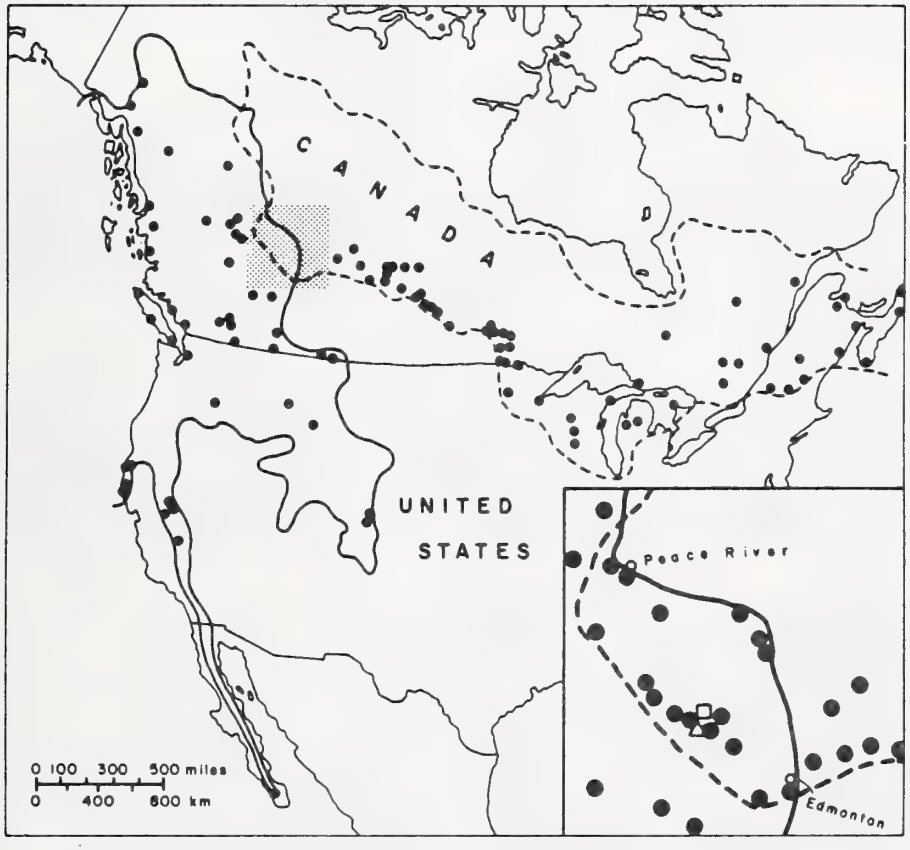


Figure 1. Distributional ranges of P. banksiana (broken line) and P. contorta (solid line). Filled circles represent populations from which cpDNA phenotypes of 363 individuals have been reported previously (Wagner et al. 1987.) Open square (Carson Creek, Alberta) indicates population of this study.

METHODS

A detailed account of both DNA extraction and the analysis of cpDNA variation in jack and lodgepole pines has been presented elsewhere (Wagner et al. 1987). The following, therefore, is a very brief description of the methods. Total cellular DNA was extracted from 10 g of fresh needle tissue. Approximately 1-2 ug of the total DNA was completely digested with the restriction endonuclease Sst I (BRL laboratories) under conditions recommended by the supplier. Restriction fragments were separated by electrophoresis and transferred to Biotrans membranes. Pine chloroplast DNA fragments were visualized by autoradiography, following hybridization (Southern 1975) of membrane-bound pine DNA to ^{32}P-labeled (Rigby et al. 1977) Petunia hybrida cpDNA (Palmer et al. 1983). Cone morphology was used to classify individuals as putative jack pine, lodgepole pine, or hybrid (Moss 1949). The association between cpDNA variation and cone morphology was tested by X^2 analysis of the contingency table (table 2).

RESULTS AND DISCUSSION

Sst I cpDNA restriction fragment banding patterns (variants) in the allopatric ranges of jack and lodgepole pines provided an unambiguous distinction between the two species in a previous allopatric sample of 202 individuals (Wagner et al. 1987). However, in this study we have detected a number of novel cpDNA variants that apparently occur only in or near the sympatric region (table 1).

Table 1.--Frequencies of cpDNA variants in the Carson Creek population.

cpDNA Varient	Frequency	Species
4.4/5.0*	42	L
4.5/5.0	230	L
4.7/5.0	23	L
4.5/4.8	1	L
4.4/5.7	2	J
4.7/5.7	6	J
4.8/5.7	15	J
5.0/5.7	0	J
Sympatric	16	
Total	335	

*Denotes Sst I fragment sizes in kilobase pairs.

These novel variants occur in approximately 5 percent of the individuals of this population, a significantly greater frequency than in the allopatric ranges ($t = 58.3$, $P < 0.0005$). The new variants might have arisen due to "bursts of transposition and mutation" (Gerasimova et al. 1984) or intragenic recombination (Golding and Strobeck 1982) in genetically unstable hybrids in the sympatric zone (Barton and Hewitt 1985). In addition, although cpDNA inheritance seems predominantly paternal in P. contorta X P. banksiana hybridizations, the novel variants may result from a low frequency of

"maternal leakage" of cpDNA. This would be the converse of the "paternal leakage" of mitochondrial genomes that has been documented in animal taxa (Powell 1983, Takahata 1985).

Using cone morphology as an indicator of the taxonomic status of each individual in the zone of sympatry, 19 individuals had cpDNA typical of Pinus banksiana with cone morphology typical of P. contorta or hybrids. Only four trees had cone morphology and cpDNA both typical of P. banksiana (table 2).

Table 2.--Association between cpDNA and cone morphology in lodgepole pine, jack pine and their hybrids in the Carson Creek population of the sympatric zone.

cpDNA	Cone Morphology		
	CONCORDANT	DISCORDANT	TOTAL
LODGEPOLE	143 (136.4)	153 (159.6)	296
JACK	4 (10.6)	19 (12.4)	23
TOTAL	147	172	319

$X^2_{[1]} = 7.01$, $P \cong 0.01$

P. contorta cpDNA, however, was associated in excess of expectations with P. contorta cone morphology. These results suggest that undirectionally biased interspecific chloroplast gene flow may occur in the sympatric zone from jack pine into lodgepole pine.

The fact that the novel variants reported here and the variants typical of jack pine or lodgepole pine (Wagner et al. 1987) do not extend beyond the zone of sympatry into the "wrong" species may be due to one of the following three factors: a) the sympatric region between jack and lodgepole pine is relatively recent and the time required for diffusion of alien chloroplast genomes across species boundaries may be long, b) the sympatric region may have been "trapped" by both genetic and environmental factors (Barton and Hewitt 1985), or c) there may be nucleo-cytoplasmic incompatibility between jack and lodgepole pines which is preventing the efficient invasion of chloroplast genomes across the species boundary.

LITERATURE CITED

Barton, N. H. and G. M. Hewitt. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst. 16:113-148.

Critchfield, W. B. 1985. The late Quaternary history of lodgepole and jack pines. Can. J. For. Res. 15:749-772.

Critchfield, W. B. 1957. Geographic variation in Pinus contorta. Maria Moors Cabot Found. Publ. 3, 118 pp.

Gerasimova, T. I., Mizrokhe, L. J. and G. P. Georgiev. 1984. Transposition bursts in genetically unstable Drosophila melanogaster. Nature 309:714-716.

Golding, G. B. and C. Strobeck. 1983. Increased number of alleles found in hybrid populations due to intragenic recombination. Evolution 37:17-29.

Kinloch, B. B., Westfall, R. D. and G. I. Forrest. 1986. Caledonian Scots pine: Origins and genetic structure. New Phytol. 104:703-729.

Ledig, F. T. 1986. Heterozygosity, heterosis, and fitness in outbreeding plants. pp 77-104, In M. E. Soule (ed.), Conservation Biology. Sinauer Associates Inc. Sunderland.

Moss, E. H. 1949. Natural pine hybrids in Alberta. Can. J. Res. Sec. C 27: 218-229.

Neale, D. B., Wheeler, N. C. and R. W. Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16:1152-1154.

Palmer, J. D. and W. F. Thompson. 1982. Chloroplast DNA rearrangements are more frequent when a large inverted repeat sequence is lost. Cell 29:537-550.

Palmer, J. D., Shields, C. R., Cohen, D. B. and T. J. Orton. 1983. Chloroplast DNA evolution and the origin of amphidiploid Brassica. Theor. Appl. Genet. 65:181-189.

Powell, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: Evidence from Drosphila. Proc. Natl. Acad. Sci. 80:492-495.

Rigby, P. W. J., Diekmann, M., Rhodes, C. and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA Polymerase I. J. Mol. Biol. 113:237-254.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Takahata, N. 1985. Introgression of extranuclear genomes in finite populations: Nucleo-cytoplasmic incompatibilty. Genet. Res. 45:179-194.

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P. and R. W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. 84:2097-2100.

Yeatman, C. W. 1967. Biogeography of jack pine. Can. J. Bot. 45:2201-2211.

Zobel, B. J. and J. T. Talbert. 1984. Applied Forest Tree Improvement. John Wiley and Sons, New York.

THE TENDENCY FOR ROUND GALL DEVELOPMENT IN LOBLOLLY PINES THAT ARE RESISTANT TO *CRONARTIUM QUERCUUM* F. SP. *FUSIFORME*

G. A. Snow[1/]

Abstract.--The hypothesis that gall shape can be an expression of resistance or susceptibility to *C.q. fusiforme* has been developed from inoculation experiments with loblolly pine seedlings. Pine families that formed long fusoid galls usually had higher percentages of trees galled than families that formed round cerebroid galls. If the relationship is the same in field-grown trees, one would expect a tendency for galls to be round in resistant populations of loblolly pine. Fusiform rust galls were examined in pine plantations in Texas, Louisiana, Mississippi, Florida, and Georgia to determine if gall shape differed by seed source and planting location. Gall length and width were measured and a gall form value (gall length/gall width) was derived for each gall. The largest values were for galls on slash pine grown from a seed source known to be susceptible to fusiform rust. Trees grown from Texas seed sources tended to have low gall form values wherever they were planted, except for some locations in Texas. Livingston Parish, Louisiana seed sources had low gall form values in all locations, except Madison County, Florida, where Livingston Parish stock is susceptible to *C.q. fusiforme*.

Gall shape may be a useful parameter in evaluating field-grown pines for rust resistance.

[1/] USDA Forest Service, Southern Forest Experiment Station, Gulfport, MS.

SEASONAL ROOTING RESPONSE OF 6-YEAR-OLD LOBLOLLY PINE CLONES TO DIFFERENT CONCENTRATIONS OF INDOLE-3-BUTYRIC ACID AND DIMETHYL SULFOXIDE

M. F. Mahalovich, S. E. McKeand, and J. B. Jett[1/]

Abstract.--Cuttings from seven clones of loblolly pine (Pinus taeda L.) were divided into five treatment groups (Hare's powder, 4000 ppm IBA + 1% DMSO, 4000 ppm IBA + 0.5% DMSO, 4000 ppm IBA and 0.5% DMSO) and rooted in a greenhouse in February, May, and September. Rooting was highest in the IBA + 1% DMSO, 0.5% DMSO and Hare's powder treatments. Highest rooting percentages occurred in February (64%) followed by September (39%) and May (29%) setting dates. Results indicate significant differences among clones and treatments in percent rooting and callus development. Significant clone*treatment and season*treatment interactions were present and suggest the need to alter rooting treatments according to the time of year.

Additional keywords: Pinus taeda, DMSO, IBA.

The focus of operational and research oriented vegetative propagation has been to develop cultural pre-treatments to promote year-round rooting of field and greenhouse cuttings or to characterize those best "rooting windows" where highest rooting percentages are realized a few months each year.

The application of dimethyl sulfoxide (DMSO) to enhance rooting of woody horticultural varieties has been summarized by Edwards (1979). DMSO, which increases the permeability of plant tissues to exogenous auxins, has been used to propagate some varieties of Juniperus (McKinniss 1969). The potential of DMSO to enhance the uptake of exogenous auxins and increase rooting in species such as loblolly pine appears to be promising. However, DMSO has not been routinely used to propagate forest tree species.

The objectives of this study with loblolly pine cuttings were: 1) to determine the effects of IBA and DMSO treatments compared to "standard" rooting treatments, and 2) to evaluate the rooting response of the selected treatments during three seasons of the year.

1/ Graduate Research Assistant, Geneticist and Assistant Professor, and Associate Director and Associate Professor, respectively, North Carolina State University-Industry Cooperative Tree Improvement Program, Raleigh, NC.

The use of trade names does not imply endorsement of products named nor criticism of products not named.

METHODS

In March, 1981, seven clones were vegetatively propagated from seedlings germinated from open-pollinated seeds in July, 1980. Rooted cuttings were transferred to 5.5 liter pots and kept hedged to a 0.25 to 0.5 m height in a greenhouse before planting in the field at the NCSU Genetics Gardens in spring 1983. Three ramets of each clone were planted and thereafter hedged annually to a height of 0.5 to 1 m.

Dormant cuttings were collected February 21, 1986. Succulent shoots were available from the trees for the May 19, 1986 collection period. A tip moth (Rhyacionia sp.) infestation limited the number of cuttings collected September 26, 1986.

Approximately 105 cuttings were collected per clone. Each cutting was submerged in a Benomyl solution (0.3 gm/l water) for one-half hour and stuck the same day in a rooting medium of a 2:2:1 ratio of perlite, vermiculite, and peat. Cuttings from the seven clones were randomly divided into five treatment groups:

(1) Hare's powder (Hare 1978)
(2) 4000 ppm IBA + 1% DMSO
(3) 4000 ppm IBA + 0.5% DMSO
(4) 4000 ppm IBA
(5) 0.5% DMSO

The IBA and/or DMSO treatments were prepared using a 50% ethyl alcohol base. Because of the limited number of cuttings available from the September collection and preliminary rooting results from the February and May experiments, some September cuttings were omitted from the 4000 ppm IBA + 0.5% DMSO treatment. Generally, there were 21 cuttings per clone per treatment for a total of 735 cuttings per experiment.

The cuttings were placed in a greenhouse where the humidity was controlled by a MEE II Cloudmaker®. Fog was maintained at a level to keep the foliage wet but without saturating the rooting medium. All cuttings were fertilized weekly with a foliar application of 15-30-15 N-P-K plus micronutrients (Peter's High Phos Special®) at 200 ppm N concentration.

Callus score (0 = no basal swelling; 1 = basal swelling, no callus; 2 = minimal callus, no roots; 3 = moderate callus with roots; and 4 = maximal callus with roots) and shoot elongation (cm) were assessed at 7 weeks. Rooting was assessed at week 12.

A two-way ANOVA was used to test for differences among clones and treatments for each rooting period. All data were analyzed on a clone-treatment-mean basis. Because clone-treatment means for percent rooting covered a wide range of values (0% - 100%), both non-transformed percentages and arc sine percentages were analyzed. No differences were noted in significance levels for the F-tests, therefore, only non-transformed percentages are presented. The three-way ANOVA used to test differences among seasons was limited to those treatments and clones common to all three rooting

periods. Significant treatment differences were evaluated using the Waller-Duncan k-ratio t-test (SAS 1985).

RESULTS

A large and significant ($p \leq 0.05$) portion of the variation in percent rooting and callus score was attributed to clones (table 1). Differences among treatments were seen in percent rooting in February and May and in callus development in February. Generally, clone and treatment differences were not important for shoot elongation.

Table 1.--Analysis of variance with significance levels for rooting percent, callus score, and shoot elongation, by month

Source of Variation	df	Mean Square[1/] Percent Rooting	Callus Score	Shoot Elongation
February				
Clone	6	0.33**	0.36**	17.49**
Treatment	4	0.05**	0.48**	0.63NS
Error	24	0.01	0.07	0.28
May				
Clone	6	0.32**	2.26**	<0.01NS
Treatment	4	0.04*	0.22NS	<0.01NS
Error	24	0.01	0.12	<0.01
September				
Clone	3	0.21**	0.79*	3.56NS
Treatment	3	0.02NS	0.23NS	0.57NS
Error	9	0.03	0.22	1.38

[1/] NS = non-significant
* = $0.01 < p \leq 0.05$
** = $p \leq 0.01$

Rooting was greatest in February, followed by September and May (figure 1). The standard rooting treatments, Hare's powder and 4000 ppm IBA, were not different from the two IBA + DMSO treatments. The addition of IBA or IBA + DMSO applied in May and September inhibited rooting, yet DMSO alone yielded the best rooting percentages at these times.

Callus development was not affected by treatment or by season when treatment was applied (figure 2). Although the presence of callus is usually necessary for root initiation in pines (Cameron 1968 and Haissig

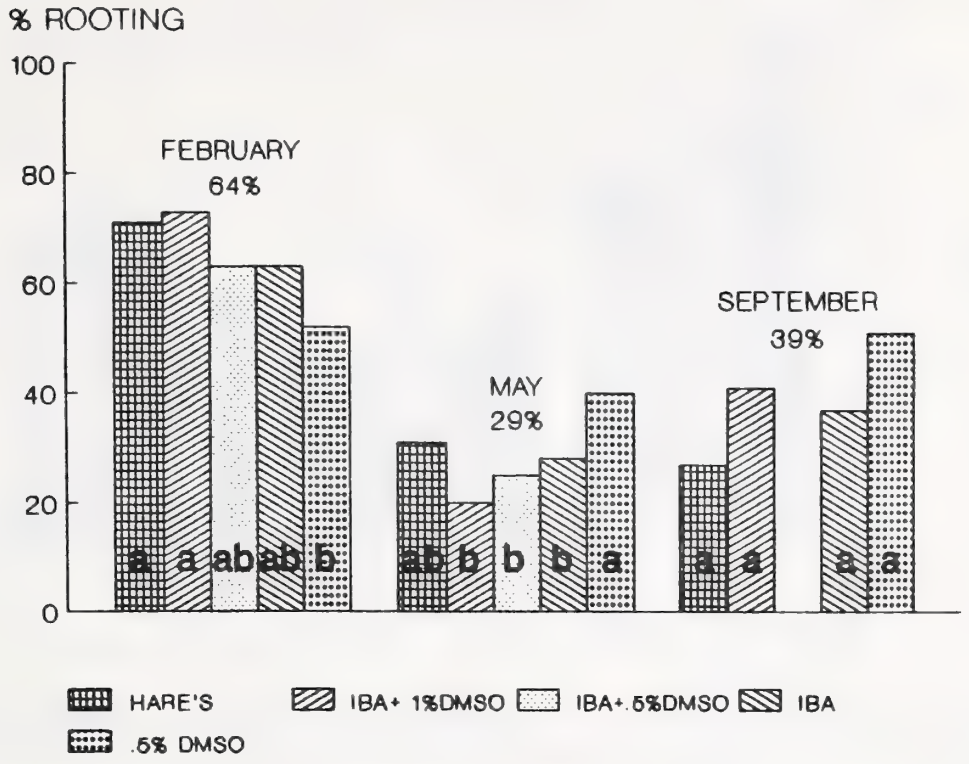


Figure 1. Seasonal trends in percent rooting of loblolly pine cuttings, by treatment. (Treatment means within a season with the same letter are not significantly different, $p \leq 0.05$).

1982), the best rooting treatments (figure 1) did not necessarily have the largest callus development at week seven (figure 2).

The treatment differences in shoot elongation were largely seasonal and were neither inhibited nor promoted by either DMSO or IBA + DMSO (figure 3). Virtually no shoot elongation was observed during May.

Because of the tip moth damage to the shoots, the overall three-way ANOVA was limited to four clones and excluded the 4000 ppm IBA + 0.5% DMSO treatment. Significant ($p \leq 0.05$) season*clone interactions were found for callus score and shoot elongation. Most importantly, season*treatment interactions were present for rooting percent and callus score (table 2).

DISCUSSION

The most important finding from this study was the seasonal trends in rooting. Rank changes in clones and treatment means were found in February and May, contributing to the clone*treatment and season*treatment interactions. The increased rooting percentages during early spring followed by early fall and late spring agree with similar treatments applied to 2- and 3-year-old slash (P. elliotti Engelm.) and loblolly pine cuttings (Reines and Bamping 1960), and 1- to 5-year-old Monterey pine (P. radiata D. Don)

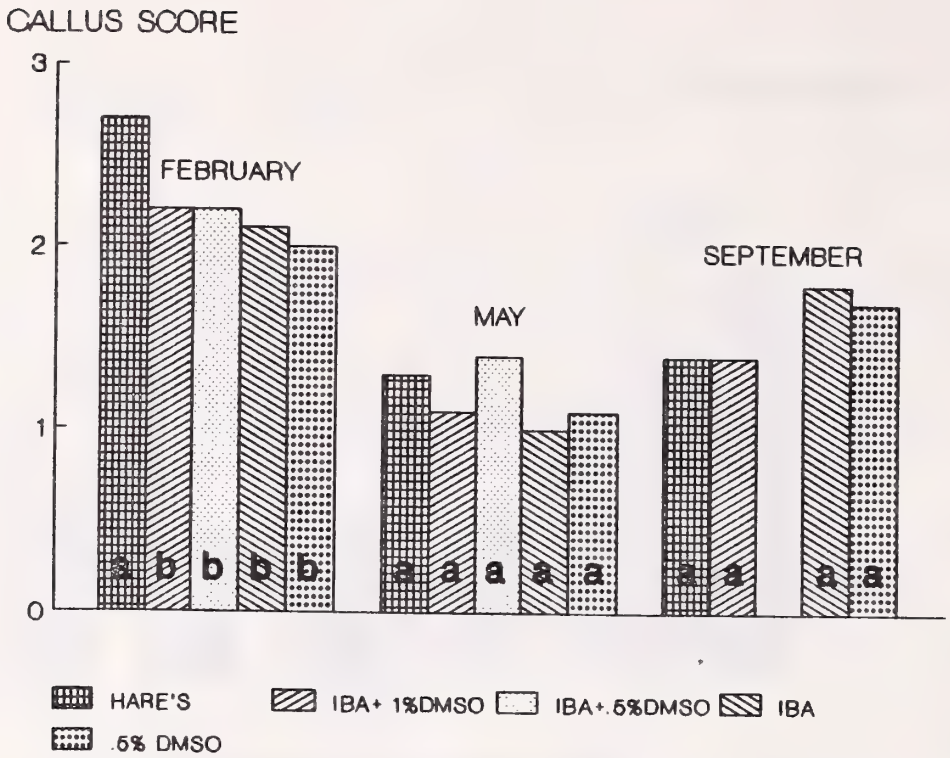


Figure 2. Mean callus score of loblolly pine cuttings by treatment. (Treatment means within a season with the same letter are not significantly different, $p \leq 0.05$.)

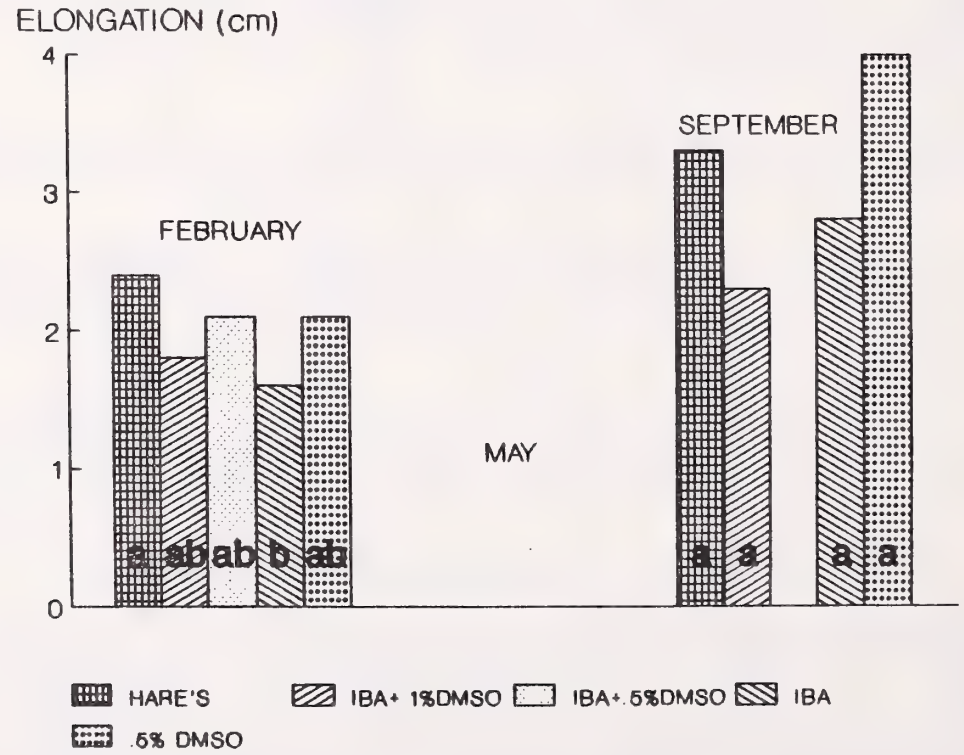


Figure 3. Mean shoot elongation of loblolly pine cuttings by treatment. (Treatment means within a season with the same letter are not significantly difference, $p \leq 0.05$.)

Table 2.--Three-way analysis of variance with significance levels for percent rooting, callus score, and shoot elongation

Source of Variation	df	Mean Square[1/] Percent Rooting	Callus Score	Shoot Elongation
Season	2	0.55**	4.43**	14.58**
Clone	3	0.44**	2.29**	14.27**
Season*Clone	6	0.02NS	0.80**	5.23**
Treatment	3	<0.01NS	0.05NS	1.32NS
Season*Treatment	6	0.03*	0.03*	1.26NS
Clone*Treatment	9	0.03*	0.17NS	0.68NS
Error	18	0.01	0.12	0.74

[1/] NS = non-significant
* = $0.01 < p \leq 0.05$
** = $p \leq 0.01$

and ponderosa pine (P. ponderosa var. arizonica (Engelm.) Shaw.) cuttings (Mahalovich, unpublished data). These results do not agree with the seasonal rooting success of slash pine (Bower and van Buijtenen 1977, Hare 1978, and Mergen 1955), where late spring and summer showed the highest rooting percentages. It is likely that the above differences can be due to species, geographic location, cultural pre-treatments, and donor age, all which affect rooting of stem cuttings.

The importance of characterizing "rooting windows" and appropriate treatments for research and operational rooting of stem cuttings cannot be ignored. As an example, percent rooting for one clone treated with IBA + 1% DMSO was 100, 52, and 100% for the February, May and September rooting periods, respectively. Rooting was poorest in May, but that clone rooted at 90% in May when 0.5% DMSO alone was used. Identifying favorable clone-treatment-season combinations will increase the number of healthy rooted cuttings which can be produced.

DMSO as an agent to transport IBA across plant tissues did not consistently enhance rooting percentages over that of Hare's powder or IBA alone. In May and September 0.5% DMSO alone had the highest rooting response. The lower percentages of the other treatments containing IBA may have been due

to some IBA toxicity. High concentrations of IBA have been shown to inhibit rooting in some species (Bowen et al. 1975, Larsen and Dingle 1969, and Whatley et al. 1966).

The role of DMSO in stimulating the rooting of pine requires further study with lower concentrations of IBA. The potential exits to use DMSO as a "carrier" of IBA into mature, woody cuttings to enhance rooting. In the absence of exogenous auxin application, pre-treatment with DMSO and ethyl alcohol may increase permeability and/or mobilize endogenous auxins thereby promoting higher rooting percentages.

LITERATURE CITED

Bowen, M. R., J. Howarth, and K. A. Longman. 1975. Effects of auxins and other factors on the rooting of Pinus contorta Dougl. cuttings. Ann. Bot. 39:647-656.

Bower, R. and R. J. van Buijtenen. 1977. A comparison of rooting success of greenhouse-grown and field-grown slash pine cuttings. Can. J. For. Res. 7(1):183-185.

Cameron, R. J. 1968. The propagation of Pinus radiata by cuttings. NZ J. For. 13(1):78-89.

Edwards, R. A. 1979. An evaluation of wounding and hormones on the rooting of cuttings (abstr.). Royal NZ Inst. Ann. J. 7:74-82.

Haissig, B. E. 1982. The rooting stimulus in pine cuttings. In: Comb. Proc. Int'l. Plant Prop. Soc. Meet. 32:625-638.

Hare, R. C. 1978. Effect of shoot girdling and season on rooting of slash pine cuttings. Can. J. For. Res. 8:14-16.

Larsen, F. E. and R. W. Dingle. 1969. Vegetative propagation of lodgepole pine (Pinus contorta Dougl.) from needle fascicles.

McKinnis, G. R. 1969. The effects of DMSO as a carrier for IBA on the rooting of three juniper species. Plant Prop. 15:4-8.

Mergen, F. 1955. Vegetative propagation of slash pine. USDA For. Ser. SE For. Exp. Stat., SE Stat. Paper #54, Asheville, NC. 61 pp.

Reines, M. and J. H. Bamping. 1960. Seasonal rooting response of slash and loblolly pine cuttings. J. For. 58(8):646:647.

SAS. 1985. SAS USER'S GUIDE: Statistics, Cary, NC, 27511.

Whatley, B. T., S. O. Thompson and G. Williams. 1966. The effects of nutrient solution, foliar spray, 3-indolebutyric acid and dimethyl sulfoxide (DMSO) on rooting of Hibiscus syriacus. In: Comb. Proc. Int'l. Plant Prop. Soc. Meet. 16:287-290.

PITCH CANKER RESISTANT SLASH PINE IDENTIFIED BY GREENHOUSE SCREENING

S. W. Oak[1/], G. M. Blakeslee[2/], and D. L. Rockwood[2/]

Abstract.--A new greenhouse-based method was used to screen for pitch canker resistance in open-pollinated slash pine families representing a range in relative resistance as determined by field tests. Four-month-old seedlings of 12 families plus a susceptible check family were inoculated by severing the stem just below the bud and spraying the cut surface with a water suspension of Fusarium moniliforme var. subglutinans conidia. Seedling responses were evaluated 3, 4, and 5 months after inoculation. Highly significant family differences were observed on all three reading dates, but the best family separation occurred 5 months after inoculation, when family mean infection exceeding the response standard ranged from 0 to 80 percent (mean 42 percent). The susceptible check was always correctly ranked. Field and greenhouse resistance class groups were nearly identical, and standardized performance scores were highly correlated. Operational greenhouse screening for pitch canker resistance appears feasible.

INTRODUCTION

Slash pine (Pinus elliottii Engelm. var. elliottii) is one of the most susceptible southern pine species to damage caused by pitch canker disease. Epidemics have periodically devastated forest plantations of this species in Florida and Georgia. The most recent widespread episode occurred during the mid-1970's, when over 1 million acres of Florida slash pine plantations were affected (Phelps and Chellman 1976), and several thousand acres were harvested prematurely to salvage damaged stands (Dwinell and Phelps 1977). Damage has also been severe in some slash and loblolly pine seed orchards (Phelps and Chellman 1976, Kuhlman et al. 1982, Kelly and Williams 1982), and in some plantations of loblolly pine (Kuhlman and Cade 1985).

Pitch canker, caused by Fusarium moniliforme Sheld. var. subglutinans Wr. & Rienk. (FMS), is a shoot-killing disease in slash pine. Losses accrue mainly from reductions in volume growth, but stem deformities sometimes occur, reducing opportunities for high-value, solid wood products (Arvanitis et al. 1984, Phelps and Chellman 1976, Schmidt and Underhill 1974). Associated mortality is usually minor, but has been known to exceed 25 percent (Blakeslee and Oak 1979).

1/ Plant Pathologist, USDA Forest Service, Forest Pest Management, Asheville, North Carolina.

2/ Associate Professors of Forest Pathology and Forest Genetics, respectively, Department of Forestry, School of Forest Resources and Conservation, University of Florida, Gainesville, Florida.

Evidence exists for variation in pitch canker resistance among slash pine genotypes (Blakeslee and Rockwood 1978, Dwinell and Barrows-Broaddus 1979, Lowerts et al. 1985, McRae et al. 1985). Disease-free trees are commonly present in devastated plantations, as well as in diseased seed orchards and progeny tests. This variation has been quantified through controlled inoculation experiments and the potential genetic gains estimated for various selection strategies. Results indicate that meaningful improvements can be made in pitch canker resistance (Rockwood et al. 1987). A rapid and reliable screening procedure for identifying resistant genotypes would greatly advance development of pitch canker resistant planting stock.

Artificial screening methods used to date to identify relative resistance in the field and greenhouse have required tedious hand wounding and inoculation procedures (Blakeslee and Rockwood 1978, Dwinell 1978, Dwinell and Barrows-Broaddus 1979, McRae et al. 1985) that are difficult to apply consistently to individual seedlings. Seedling morphology, wound size and depth, the types of tissues exposed, and inoculum exposure can vary considerably within and between tests. A new screening method that can be more quickly and uniformly applied was tested to determine if differences in relative resistance could be detected for a group of slash pine families and if the results were comparable to those obtained from field progeny tests.

MATERIALS AND METHODS

Twelve half-sib slash pine families were selected for the greenhouse test. Based on their responses in one or more of four artificially inoculated tests of field-grown trees ranging in age from 3 to 22 years, these families represented a range in relative pitch canker resistance (White et al. 1987). Selected families fell into three relative resistance groups (resistant, intermediate, and susceptible); relative resistance classifications were identical for families common to different tests. After the greenhouse test was initiated, information from an additional artificial inoculation field test and a naturally infected field test became available. Results from these tests were also used in subsequent comparisons of field and greenhouse resistance. The 12 families were given arbitrary letter codes (A-L), and the greenhouse test was conducted without knowledge of the family identities or relative resistance classification. An additional susceptible check family (B2) was also included. Selection of this check family was based on our (Oak and Blakeslee) knowledge of its propensity for severe damage in infected seed orchards and in past greenhouse inoculation experiments.

Prior to inoculation, the seedlings were grown in Ray Leach Super Cells for 4 months in a greenhouse at the USDA Forest Service Resistance Screening Center (RSC) near Asheville, NC. When they were inoculated, seedlings in all families had similar morphology. Stems were succulent with only primary needles, basal diameter averaged 2 to 3 mm, and heights averaged 75 to 100 mm. The seedlings were inoculated by severing the stem just below the bud and spraying the exposed cross-section with a water suspension of FMS conidia (1×10^5 conidia/ml) until visibly wet. The inoculum was an equal mixture of three separate proven pathogenic isolates originating from diseased slash pines growing in Liberty County, Florida. It was prepared in the manner described by McRae et al. (1985).

The experimental design was identical to that used for fusiform rust screening at the RSC (Anderson et al. 1983). Three trays of 20 seedlings of each family were inoculated on each of 2 consecutive days. At 3, 4, and 5 months after inoculation, the percentage of available stem length (cut surface to cotyledons) killed by FMS was estimated for each seedling. Two response standards were used--50 and 75 percent of available stem length killed. The percentage of trees that exceeded the response standard in each tray was subjected to analysis of variance procedures to determine family, run, and family-by-run (experimental error) interactions. Duncan's Multiple Range test was used to compare family test means.

The relationship between greenhouse and field test performance was statistically evaluated using rank and product-moment correlations. Family means in field and greenhouse tests were converted to standardized scores by subtracting the test mean from the family mean and dividing by the square root of the error mean square. Correlations were based on 9 of the 12 families for which field tests permitted standardized scoring.

RESULTS AND DISCUSSION

As FMS colonized the succulent shoots, the tissues turned purplish and resin was exuded. Symptoms of infection were evident within 1 month after inoculation, but they were relatively uniform within and among families, being restricted to less than about 1.5 cm of the stem. By 3 months, considerable variation in individual seedling response was evident. Symptoms ranged from just a few millimeters of killed stem to seedling mortality. Adventitious shoots that formed after wounding were frequently killed as the fungus advanced down the stem.

A broad range of family means (percent of seedlings exceeding the response standard) occurred at all reading dates and for both response standards. For the 75 percent response standard, the overall test mean increased nearly 20 percent (from 17 to 35 percent) between the 3- and 5-month readings, while the overall test mean using the 50 percent standard increased 10 percent over the same period (32 to 42 percent). The 5-month reading of the 50 percent response standard provided the best differentiation of family performance, with individual family means ranging from 0 to 80 percent.

Analysis of variance indicated highly significant family differences for both response standards at all reading dates. Table 1 illustrates the results for the 50 percent response standard at 5 months. Significant family-by-run interactions were absent.

Statistical separation of family means allowed grouping of families into relative resistance classes according to the greenhouse test results (Table 2). The susceptible check was the most susceptible family and was correctly ranked in all analyses.

Table 1.--Analysis of variance for mean pitch canker infection response of 12 half-sib slash pine families and a susceptible check for the 50 percent response standard, 5 months after inoculation in the RSC greenhouse test.

Source of Variation	DF	S.S.	M.S.	F.
Families	12	38560.25	3213.35	36.54**
Runs	1	566.69	566.69	6.44*
Families x Runs	12	1055.37	87.95	.97
Among Replicate Trays within Runs	52	4708.19	90.54	

** and * denote significance at the 1% and 5% levels, respectively.

The relative pitch canker resistance classes determined in the greenhouse testing closely agreed with resistance class groupings based on the field tests (Table 2). Only one family (F) did not fall into the same resistance class group in the greenhouse as it had in an earlier field test. The method of pathogen introduction apparently has little influence on detecting relative resistance. The resistance class groupings for four families (B, D, H, I) common to the naturally infected field test, two artificially inoculated field tests (H only one), and the greenhouse test were identical.

One of the 12 test families (B) represented a different collection (different orchard) of the same family used as the susceptible check (B2). Both of these families were grouped in the same relative resistance class in the greenhouse test and in the field tests, but they received statistically different specific rankings (10th and 13th) according to family mean infection in the greenhouse test.

The strong correspondence between the greenhouse and field results suggested by the similar family resistance class groupings was further supported by significant correlations between standardized scores. For the nine families having both field and greenhouse scores, both the rank correlation (.85) and the product-moment correlation (.845) were significant at the 1 percent level.

While the similarities in resistance class groupings and significant correlations between greenhouse and field test scores are encouraging, it must be recognized that this test provides only a single comparison between a relatively small number of families. Additional experiments are underway that will: 1) evaluate the repeatability of this test with the same families plus an additional group of families that represent a range in relative resistance,

Table 2.--Mean percent pitch canker infection, relative pitch canker resistance classes, and standardized scores for 12 half-sib slash pine families, based on field and greenhouse testing.

Family	Field Tests		Greenhouse Test		
	Resistance[1] class	Current[2] standardized score	Family[3] mean	Resistance[4] Class	Standardized[5] Score
D	R (3)	1.18	0 a	R	1.83
G	R (1)		13 b	R	1.21
I	R (3)	2.07	17 b	R	1.02
J	R (1)	1.30	23 b	R	.74
F	S (1)		38 c	I	.02
K	I (1)		43 c	I	- .21
E	I (1)	- .49	43 c	I	- .21
C	I (2)	- .04	48 cd	I	- .45
L	I (3)	- .13	49 cd	I	- .50
B	S (3)	- .34	58 de	S	- .93
H	S (2)	-1.18	63 e	S	-1.16
A	S (1)	-3.16	67 e	S	-1.35
Suscept. Check - B2			80 f		

[1]Relative pitch canker resistance class (R=resistant, I=intermediate, S=susceptible) based on performance in artificially and/or naturally infected field tests conducted independent of greenhouse test. ( ) indicates number of field tests from which classifications were made.

[2]Current standardized performance score (standard deviations from the overall test mean) based on performance in an artificially or naturally inoculated field test with 40 or more families and more than 30% infection.

[3]Duncan's Multiple Range Test for mean pitch canker infection (50% percent response standard at 5 months). Test means followed by different letters are significantly different at the 5% level.

[4]Relative pitch canker resistance class (R=resistant, I=intermediate, S=susceptible) based on response in RSC greenhouse test.

[5]Standardized performance score (standard deviations from the overall test mean) based on response in RSC greenhouse test.

and 2) evaluate the feasibility of pitch canker resistance screening in loblolly pine. Favorable results may lead to operational pitch canker screening at the RSC.

CONCLUSIONS

The stem severance-inoculation procedure reported in this study accurately detected differences among slash pine families that represent a broad range of relative resistance to pitch canker. This method is quicker, easier, and may be more consistently applied than current screening procedures. The relative resistance class groupings and infection rankings were consonant with existing knowledge of field performance. These results suggest that pitch canker resistance in slash pine may be routinely and reliably evaluated by this greenhouse procedure.

LITERATURE CITED

Anderson, R. L., C. H. Young, and J. D. Triplett. 1983. Resistance Screening Center procedures manual: a step-by-step guide used in the operational screening of southern pines for resistance to fusiform rust. USDA For. Serv., For. Pest Manage., Field Office Rep. 84-1-1. 55 pp.

Arvanitis, L. G., J. F. Godbee, Jr., and I. Porta. 1984. Pitch canker impact on volume growth: a case study in slash pine plantations. S. J. Appl. For. 8:43-47.

Blakeslee, G. M. and D. L. Rockwood. 1978. Variation in resistance of slash pine to pitch canker caused by Fusarium moniliforme var. subglutinans. (Abstr.) Phytopath. News 12:207-208.

Blakeslee, G. M. and S. W. Oak. 1979. Significant mortality associated with pitch canker infection in slash pine in Florida. Plant Dis. Rep. 63:1023-1025.

Dwinell, L. D. and W. R. Phelps. 1977. Pitch canker of slash pine in Florida. J. For. 75:488-489.

Dwinell, L. D. 1978. Susceptibility of southern pines to infection by Fusarium moniliforme var. subglutinans. Plant Dis. Rep. 62:108-111.

Dwinell, L. D. and J. Barrows-Broaddus. 1979. Susceptibility of half-sib families of slash and loblolly pine to the pitch canker fungus, Fusarium moniliforme var. subglutinans. (Abstr.) Phytopathology 69:527.

Kelly, W. D. and J. C. Williams. 1982. Incidence of pitch canker among clones of loblolly pine in seed orchards. Plant Disease 66:1171-1173.

Kuhlman, E. G., S. D. Dianis, and T. K. Smith. 1982. Epidemiology of pitch canker disease in a loblolly pine seed orchard in North Carolina. Phytopathology 72:1212-1216.

Kuhlman, E. G. and S. C. Cade. 1985. Pitch canker disease of loblolly and pond pines in North Carolina plantations. Plant Disease 69:175-176.

Lowerts, G. A., M. H. Zoerb, Jr., and J. Pait. 1985. Resistance to the development of pitch canker in open-pollinated slash pine families. In: pp. 334-340, Proc. 18th S. For. Tree Imp. Conf., Long Beach, MS.

McRae, C. H., D. L. Rockwood, and G. M. Blakeslee. 1985. Evaluation of slash pine for resistance to pitch canker. In: pp. 351-357, Proc. 18th S. For. Tree Imp. Conf., Long Beach, MS.

Phelps, W. R. and C. W. Chellman. 1976. Evaluation of "pitch canker" in Florida slash pine plantations and seed orchards - 1976. USDA For. Serv., Southeast. Area, State and Private For. 22 pp.

Rockwood, D. L., G. M. Blakeslee, G. A. Lowerts, E. M. Underhill, and S. W. Oak. 1987. Genetic strategies for reducing pitch canker incidence in slash pine. (In press).

Schmidt, R. A. and E. M. Underhill. 1974. Incidence and impact of pitch canker in slash pine plantations in Florida. Plant Dis. Rep. 58:451-454.

White, T., G. Hodge, D. Rockwood, H. Kok, G. Powell, M. Flinchum, J. Sibley, D. Dippon, and G. Blakeslee. 1987. Coop. For. Gen. Res. Progm. 29th Prog. Rep., SFRC Res. Rep. No. 38. University of Florida. 54 pp.

SILICON EFFECTS ON LOBLOLLY PINE SEEDLING GROWTH AND WATER STATUS

S. F. Emadian and R. J. Newton[1]

Abstract.--Silicon (Si) is found deposited within various tissues of many plants, including gymnosperms. The objectives of this investigation were: 1) to quantify Si accumulation in loblolly pine (Pinus taeda L.), and 2) to determine the effects of Si on growth, seedling water loss and water status concurrent with an induced water stress.

Silicon was administered to potted seedlings growing in a growth chamber as K_2SiO_3 (Si concentration = 0, 130, 389, and 648 nM) as a nutrient solution. Applications were either once or twice a week. Atomic absorption analysis revealed a three-fold accumulation for the fascicles of Si-treated seedlings compared to their controls.

Water stress was induced by withholding the water/nutrient solution. Silicon treatment stimulated: 1) increases in fascicle diffusive resistance, 2) decreases in transpiration rates, and 3) increases in fresh weight in water-stressed seedlings. Pressure-volume isotherm analysis showed that Si increased tissue elasticity in both water-stressed and non-stressed seedlings and symplastic weight of water in stressed seedlings. It was concluded that Si-induced increases in loblolly pine seedling growth may be the result of reduced water loss and increased tissue elasticity during water stress.

Additional keywords: silicon, growth, diffusive resistance, transpiration, elasticity.

Silicon (Si) deposition in plants has been reported for both herbaceous and woody species, in epidermal and endodermal tissue and within the vessels of shoots and roots (Yoshida and Kitagishi 1962, Jones and Hendreck 1967, Scurfield et al 1974). Silicon enters plants as a component of water-soluble monosilicic acid, H_4SiO_4, which is transported subsequently through the root, stem and leaves (Barber and Shone 1966). Aston and Jones (1976) noted that the sites and pathways of transpiration were sites of Si accumulation. They observed large amounts of Si around guard cells and subsidiary cells.

Werner and Ross (1983) reported that gymnosperms accumulate large quantities of Si. Other researchers have suggested a beneficial role for Si relative to growth (Lewin and Reiman 1969, Yoshida and Kitagishi 1962, Miyake and Takahashi 1978, Adatia and Besford 1986), water economy and/or drought resistance (Yoshida and Kitagishi 1962). Adatia and Besford (1986) observed increased fresh and dried weights of cucumber plants grown hydroponically in a Si-amended nutrient solution. Yoshida and Kitagishi (1962) found that

---

[1]Graduate Student and Associate Professor, Dept. of Forest Science and the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843.

transpiration in Si-sufficient rice plants was 30% less than in Si-deficient plants.

The physiological role of Si is not well established. Its function in plant growth and transpiration, especially for gymnosperms, needs clarification.

The objectives of this study were: 1) to measure Si in fascicle tissue of loblolly pine (Pinus taeda L.) which was absorbed from the nutrient media, and 2) to relate Si deposition in fascicles to growth, seedling water loss and tissue water status.

MATERIALS AND METHODS

BA3R13-41 originated from a typically xeric habitat in Texas, and M-2-C was selected from a mesic habitat in Mississippi. Seeds were first stratified for 30 to 45 days and planted either in 25 cm Ray-Leach containers containing medium-textured sand and located in a greenhouse, or in 7.5 x 7.5 x 25 cm Marx-5, Spenser Lamier single containers, containing fritted clay, and located in a growth chamber. Distilled water was applied to the containers every day until the seedlings produced their primary fascicles; thereafter, a nutrient solution was applied every day for five days. On the remaining two days of the weeks, distilled water was applied to avoid accumulation of mineral salts in the growing media. Seedlings in the sand medium were maintained in a greenhouse in this manner for nine months, and then transferred to the growth chamber. Seedlings in the fritted clay medium were maintained in the growth chamber from the beginning. The growth chamber had a photoperiod of 12 hours and a constant temperature of 25°C. Light intensity was 350 $\mu E\ m^{-2}\ sec^{-1}$.

After four to six weeks in the growth chamber, Si treatments were initiated. Silicon, as K_2SiO_3 was added to the nutrient solution in concentrations of: 0, 130, 389, and 648 nM. Seedlings were treated once or twice a week at the respective concentrations prior to induction of water stress. Silicon applications were continued until experiments were concluded. Water stress was induced by withholding water for a period of either 4 days (for seedlings growing in sand) or 12 to 14 days (for seedlings growing in fritted clay). Each drying cycle was terminated when predawn fascicle water potential (Ψ_w) of the representative seedlings reached -1.2 MPa. Fascicle Ψ_w was determined using a pressure chamber. Drying cycles were repeated for a period of 5 to 26 weeks. At the end of each drying cycle, all seedlings were watered with nutrient solution, with or without the addition of Si, until the growth medium was fully saturated and the predawn Ψ_w reached -0.2 MPa.

Growth was determined by measuring turgid-fresh weight (W_o) of shoots and roots immediately before pressure-volume (P-V) analysis. Shoot and root dry weight (W_d) was determined by oven drying at 105°C. Diffusive resistance (r_w) and transpiration rates (T) were determined using a Li-Cor Steady State Porometer (Model LI-1600). Four seedlings with two fascicles/seedling were used for both r_w and T measurements. Measurements were begun 4 hours after initiation of the photoperiod and were taken at 1, 2, 3, and 4 days after watering. The weight-averaged bulk modulus of elasticity ($\bar{\epsilon}$) and the symplastic weight of water (W_s) were determined by analyzing P-V isotherms (Emadian 1987). Silicon content in whole fascicles was determined using an atomic absorption spectrophotometer (Perkin Elmer, Model 603).

RESULTS AND DISCUSSION

Electron microscopy and x-ray analysis revealed that Si was deposited, exclusively within the epidermal cells of Si-treated fascicles, as clusters of small dark particles (Emadian 1987). Similar deposits were not observed in comparable tissues of control seedlings. Atomic absorption analysis revealed that the Si-treated fascicles accumulated 1.6 mg/gDW of Si, about three times more Si than did controls (0.5 mg/gDW) (Table 1). Presumably, the Si in fascicles of control plants was due to Si released from the sand, the media used for growing the seedlings.

Table 1. Silicon content (mg/g dry weight) in fascicles[1] from 18-month old loblolly pine seedlings.

Paired Sample	648 nM Si	Control (Si)
1	1.7	0.6
2	1.4	0.5
3	1.8	0.5
4	1.3	0.5
Mean	1.6a	0.5b

[1]Each sample consisted of 1 g dried tissue. p = 0.05 using Duncan's Multiple Range Test. Treatments with the same letter in common are not significantly different.

Silicon increased both W_o and W_d of loblolly pine seedlings (Table 2). Although growth increases were only 6% for well watered seedlings, growth increased 32 to 36% when Si-treated seedlings were water-stressed.

Table 2. Growth parameters[1] for 5-month old loblolly pine seedlings grown in a Si-amended fritted clay medium and subjected to water stress.

Treatment	Fresh shoot weight (W_o, g)	Dry shoot weight (W_d, g)	Dry root weight (W_d, g)
+Si -WS	17.1a	4.6a	1.8a
-Si -WS	15.5a	4.1a	1.7a
+Si +WS	11.0b	3.0b	1.1b
-Si +WS	8.1c	2.2c	0.8c

[1]Values are means of 10 replicates. p = 0.5 using Duncan's Multiple Range Test. Treatments with the same letter in common are not significantly different. All comparisons are within columns.

Diffusive resistance was increased for all concentrations of Si and frequency of its application, and such increases were more pronounced as water stress developed further in the seedlings. An opposite pattern was observed

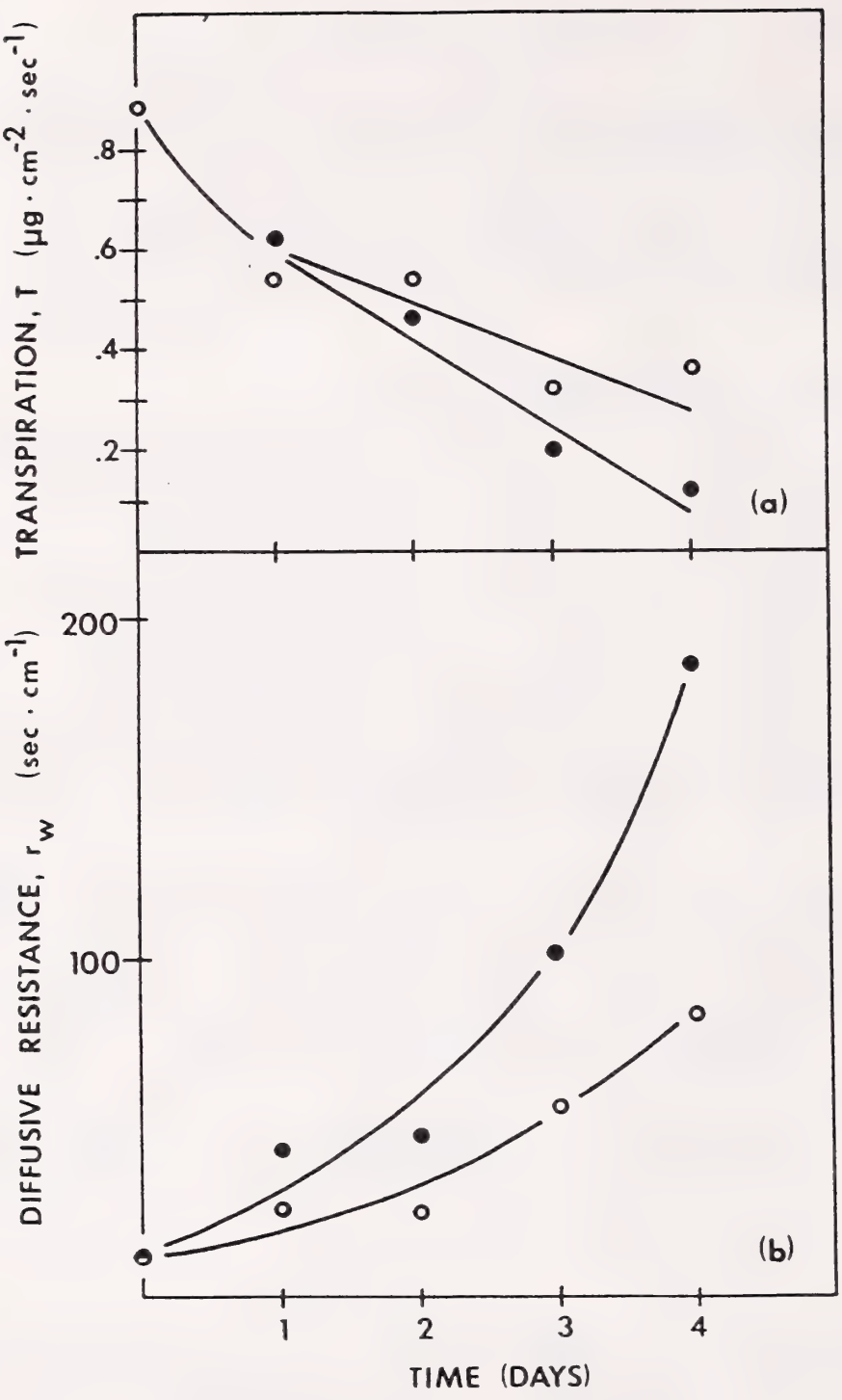


Figure 1. (a) Relationship between fascicle transpiration rate (T) of loblolly pine seedlings and time after watering with Si-amended nutrient solution for six months. (b) Relationship between fascicle diffusive resistance (r_w) of loblolly pine seedlings and time after watering with Si-amended nutrient solution for six months. •----• 648 nM Si, twice per week; o----o control, 0 nM Si, twice per week. Seedlings growing in a sand medium.

with T (Figure 1). By the fourth day after watering, T was decreased more than 75% and r_w was increased 125% compared to controls.

Silicon at 648 nM, applied twice per week, increased shoot elasticity (reduced $\bar{\epsilon}$) compared to controls (Table 3). This occurred under both stressed and non-stressed conditions. Oppositely, water stress decreased shoot elasticity (increased $\bar{\epsilon}$) in the presence or absence of Si (Table 3). Silicon increased the symplastic weight of water (W_s) only in stressed seedlings whereas, water stress decreased W_s in both Si-treated and control seedlings (Table 3).

Table 3. Seedling water status parameters derived from pressure-volume isotherms after treatment with Si and/or water stress.

Treatment	Weight-average bulk modulus of elasticity ($\bar{\epsilon}$, MPa)	Symplastic weight of H_2O (W_s, g)
+Si -WS	3.9c	5.2a
-Si -WS	5.3b	5.2a
+Si +WS	4.7bc	4.0b
-Si +WS	6.4a	3.1c

p = 0.05 using Duncan's Multiple Range Test. Treatments with the same letter are not significantly different; all comparisons made within columns.

Although Si has not been conclusively established as an essential element, it is clearly beneficial to the growth of loblolly pine. Under an induced water stress, in a Si-amended soil, seedlings are better able to retain a greater propensity for increases in fresh weight growth. Increased seedling fresh weight appears to be related to increases in symplastic weight and reduced fascicle water loss during stress. Silicon-induced increases in shoot elasticity may also be one of the mechanisms by which increased symplastic weight of water is accomplished. More elastic tissues coupled with reduced water loss could result in larger cell volumes and larger overall fresh weight. Therefore, the long established preference of loblolly pine for sandy soils may well be due in part to the physiological role of Si. Furthermore, this study suggests that Si treatment of seedlings might provide enhanced survivability on drought-prone sites.

LITERATURE CITED

Adatia, M.H., and R.T. Besford. 1986. The effects of silicon on cucumber plants grown in recirculating nutrient solution. Ann. Bot. 58:343-351.

Aston, M.J., and M.M. Jones. 1976. A study of the transpiration surfaces of Avena sterilis L. var. Algerian leaves using monosilicic acid as a tracer for water movement. Planta 130:121-129.

Barber, D.A., and M.G.T. Shone. 1966. The absorption of silica from aqueous solution by plants. J. Exp. Bot. 17:569-578.

Emadian, S.F. 1987. Physiological responses of loblolly pine (Pinus taeda L.) to silicon and water stress. Ph.D. dissertation. Texas A&M University, College Station, TX.

Jones, L.H.P., and K.A. Hendreck. 1967. Silica in soils, plants and animals. Adv. Agron. 19:107-149.

Miyake, Y., and E. Takahashi. 1978. Silicon deficiency of tomato plants. Soil Sci. Plant Nutr. 24:175-189.

Scurfield, G., C.A. Anderson, and E.R. Segnit. 1974. Silica in woody stems. Aust. J. Bot. 22:211-229.

Yoshida, S., and K. Kitagishi. 1962. Histochemistry of silicon in the rice plant. III The presence of a cuticle-silica double layer in the epidermal tissue. Soil Sci. Plant Nutr. Tokyo 8:1-5.

USE OF EXCESSIVE WATERLOGGING AND PHYSIOLOGICAL RESPONSES TO MEASURE GENETIC VARIATION IN LOBLOLLY PINE WATERLOGGING TOLERANCE

T. H. Shear and D. D. Hook [1/]

Abstract.-- Eleven sources of loblolly pine were studied for genetic variation in waterlogging tolerance and physiological responses to waterlogging. There were source differences in both though there were no correlations between them.

Additional keywords: Pinus taeda, progeny tests, flooding, anaerobic metabolism

The purposes of this study were to determine: a) if there was interspecific genetic variation in waterlogging tolerance among several sources of loblolly pine (Pinus taeda L.); b) if this variation could be determined earlier under controlled excessive waterlogging stress than in field plantings, and; c) if some of the physiological bases of this variation could be determined. The rationale and significance of these investigations have been discussed earlier (see Hook and Shear in these proceedings) and will not be reiterated here.

MATERIALS AND METHODS

Eleven sources of loblolly pine were studied, including:

1. Seven half-sibling families from two commercial seed orchards. The five families from the Federal Paperboard Co. orchard in Lumberton, NC (designated 6-13, 504, 512, 529, and 530) were originally selected as good performers on wet sites. The two families from the Westvaco seed orchard (11-9 and 7-56) in Ravenel, SC grow well throughout the coastal plain of South Carolina.

2. Three half-sibling families from single isolated trees growing in cypress-tupelo swamps in the coastal plain of South Carolina (HB, BB, and RS). Individual trees or small groups of loblolly pine often occur in these areas where they grow very well.

3. The general seed source used by the South Carolina Forestry Commission nursery in St. George, SC (STG). This source is a combination of many open-pollinated half-sibling families.

Seedlings from each source were planted on three field sites of varying wetness on the Francis Marion National Forest in SC in June, 1984. The design of each test is slightly different, though each consists of seven-tree row plots in varying numbers of replications and blocks, as described in Table 1.

[1/] Postdoctoral Research Associate and Professor and Director, respectively, Forested Wetlands Research Program, Forestry Sciences Laboratory, Clemson University, 2730 Savannah Highway, Charleston, SC 29407.

Table 1. Description of wet sites and test designs. Each replication contains a seven-tree row plot of each source.

Site Name	Mean Water Table Depth cm. Winter 1985-6	Summer 1986*	Test Design
Yellow Jacket Road (wet)	11.1	23.1	2 blocks, 4 reps each
Horse Island Road (wetter)	+0.6**	24.4	3 blocks, 2 reps each
Farewell Corner (wettest)	+13.1	14.2	1 block, 5 row plots of each source

* one of the driest seasons on record
** values preceded by a + indicate height of water above the soil surface

Seedlings were also planted at the same time in six soil tanks in which the water table level could be controlled. The tanks were filled with a Bethera series soil from the Francis Marion National Forest that will not support good pine growth when waterlogged without phosphorus fertilization. 50 ppm of phosphorus as monocalcium phosphate was added to the soil and a twelve-tree row plot of each source was planted in each tank. Three tanks were continuously waterlogged with the water table at the soil surface. The other tanks were waterlogged from November to April, but the water table was held at about 30 cm below the soil surface during the remainder of the year.

Growth measures were taken on all trees at the end of each growing season. At the end of the third growing season (1986), the seedlings in the soil tanks were harvested, oven-dried, and weighed. Fresh root tips were collected from most trees and for analyzed for: a) alcohol dehydrogenase (ADH) activity by the method of Denslow and Hook (1986), and b) anaerobic carbon dioxide (CO_2) production and resultant ethanol production by the methods of Hook and Denslow (1986). There were few viable root tips from each seedling, so either ADH activity or anaerobic metabolism (CO_2 and ethanol production) were measured, but seldom both.

Finally, standard regression and analysis of variance techniques were used to examine and compare variations in growth and physiological measures.

RESULTS AND DISCUSSION

After three growing seasons, differences in growth were developing at the wettest field site (Table 2). However, there were no significant differences in growth among sources at the other two field sites. Within family variations were very large, however, while all sources had many small trees, all did not have tall trees. The smaller trees have been dying and if they continue to do so, significant differences among sources should develop. The sources changed rank after every growing season. However, there was some stabilization and some sources were consistently good performers in all tests while others were consistently poor. Ranks in the three wet sites and the periodically waterlogged test were correlated, however, the results of the continuously waterlogged tests were poorly correlated with the other tests. Generally, the Federal Paperboard sources ranked higher when continuously

waterlogged while the Westvaco and swamp sources ranked lower. FP 512 had the largest height growth under continuous waterlogging, but grew poorly in all other tests. We will continue to measure the field tests annually to determine if long term field performance is related to short term performance under controlled waterlogging.

There were significant differences in ADH activity and ethanol production among sources in the periodically waterlogged test, but no differences in CO_2 production (Table 3). Conversely, in the continuously waterlogged test, levels of CO_2 production differed, but there were no differences in ADH activity or ethanol production. The levels of ADH activity were similar among treatments, but carbon dioxide and ethanol production were much higher under continuous waterlogging. This suggests that there are differences between the short term and long term responses to waterlogging. However, none of the physiological measures were related to growth or performance.

Collecting adequate numbers of physiological measures in a reasonable time is a difficult task. Only seven samples of fresh root tips could be tested in a single day for ethanol and CO_2 production using a Warburg gas measurement apparatus. As a result, the soil tanks were harvested over a two-month period. These physiological variables change with stage of development, growth phase, and many other factors, and surely changed over the harvest. Also, the unharvested trees continued to grow. The day of harvest was included in all models and was an important variable. However, we are currently developing gas chromatographic techniques that will allow us to process a greater number of smaller samples and reduce the variance introduced by an extended harvest. Also, by reducing the number of root tips in each sample, we will be able to measure a greater number of physiological parameters on each tree.

Since all progeny were from open-pollinated seeds, we could not control or measure the variation introduced by the paternal parent. While there were not significant differences in growth in all tests, all sources produced many small trees but only some of the sources made large trees.

While survival was good in both tank treatments, the poorest trees had died by the time of harvest. We do not know what they did or did not do that resulted in intolerance to waterlogging (or other causes of death).

Yet, despite these limitations, we have demonstrated genetic differences in both growth and physiological responses. We are currently conducting similar tests in growth chambers and with rooted cuttings in pots in greenhouses. We are confident that by taking more physiological measures in a shorter time, controlling sources of geographical and parental variation, and using techniques of plant growth analysis, we will begin to uncover the fundamental mechanisms of genetic variation in waterlogging tolerance.

Acknowledgement: This research was supported in part by the USDA Competitive Research Grant Program, Grant Number 85-08335.

LITERATURE CITED

Denslow, S., and D. D. Hook. 1986. Extraction of alcohol dehydrogenase from fresh root tips of loblolly pine. Can. J. For. Res. 16:146-148.

Hook, D. D. , and S. Denslow. 1986. Variation in physiological responses of four loblolly pine families to two water regimes. In: Plant Life in Aquatic and Amphibious Environments. R. M. M. Crawford, ed., British Ecological Society Special Symposium, Blackwell, Oxford, 1986.

Table 2. Means of growth and physiological parameters and ranks by source after three growing seasons. Means followed by the same letter were not determined to be different by Tukey's studentized range test (alpha=0.05; no letters are given for a test if there were no differences).

	Height (cm)					Total Weight (gm)		Per			Con		
Source	YJ *	HI	FC	Per	Con	Per	Con	ADH	CO2	EtOH	ADH	CO2	EtOH
BB	139	137	72 ab	118	46	80 b	19 ab	.3129 ab	.0391	.30 ab	.4298	.0617 ab	.47
HB	127	146	76 ab	126	47	72 b	12 a	.4702 ab	.0297	.35 ab	.4559	.1154 a	.51
RS	135	134	78 ab	129	46	91 ab	18 ab	.4036 ab	.0266	.37 ab	.3674	.0484 b	.51
STG	138	149	79 ab	130	51	120 a	22 b	.4615 ab	.0179	.24 b	.4546	.0651 ab	.53
FP6-13	120	122	69 b	113	54	74 b	22 b	.4856 ab	.0174	.37 ab	.3807	.0795 ab	.48
FP504	130	123	65 b	117	49	92 ab	22 b	.3605 ab	.0379	.48 a	.3191	.0052 b	.45
FP512	149	143	87 a	126	54	84 b	21 ab	.4972 b	.0429	.28 ab	.4246	.0568 ab	.41
FP529	136	130	78 ab	123	51	75 b	19 ab	.4687 ab	.0429	.36 ab	.3949	.0696 ab	.57
FP530	141	137	83 ab	117	51	86 ab	22 b	.3989 ab	.0322	.43 ab	.4054	.0685 ab	.55
WV11-9	140	134	80 ab	126	49	85 ab	21 b	.2838 a	.0389	.29 ab	.4100	.0619 ab	.46
WV7-56	142	149	90 a	129	49	88 ab	15 ab	.4103 ab	.0293	.43 ab	.4863	.0700 ab	.42
all	136	137	78	123	50	86	19	.4139	.0323	.35	.4117	.0638	.49
Rank in above test (1 = smallest, 11 = largest)													
BB	2	9	4	6	3	4	4	2	9	4	8	4	5
HB	7	7	3	4	1	1	1	9	5	5	10	11	7
RS	4	4	6	9	2	9	3	5	3	8	2	1	8
STG	6	10	7	11	9	11	11	7	2	1	9	6	9
FP6-13	3	2	1	2	5	2	8	10	1	7	3	10	6
FP504	11	8	10	7	10	10	10	3	7	11	1	2	3
FP512	1	1	2	1	11	5	7	11	11	2	7	3	1
FP529	9	6	9	3	7	3	5	8	10	6	4	8	11
FP530	5	3	5	5	8	7	9	4	6	9	5	7	10
WV11-9	8	5	8	8	4	6	6	1	8	3	6	5	4
WV7-56	10	11	11	10	6	8	2	6	4	10	11	9	2

* Abbreviations: YJ - Yellow Jacket Road test (wet site); HI - Horse Island Road test (wetter site); YJ - Corner test (wettest site); Per - periodically waterlogged soil tank test; Con - continuously waterlogged soil tank test; ADH - alcohol dehydrogenase activity ((umoles/mg root tip/minute)/10); CO2 - anaerobic carbon dioxide production ((umoles/mg root tip/hour)/100); EtOH - anaerobic ethanol production ((umoles/mg root tip/hour)/1000)

Table 3a. Correlation matrix of means of growth and physiological measures (eleven sources; r greater than .59 significant when alpha = 0.05).

	HI-HT*	FC-HT	PER-HT	C-HT	PER-WT	C-WT	P-ADH	P-CO	P-ETOH	C-ADH	C-CO	C-ETOH
YJ-HT	.54	.76	.47	.05	.30	.11	-.19	.51	-.30	.38	-.19	-.26
HI-HT		.72	.74	-.16	.37	-.49	.18	-.12	-.41	.89	.45	-.10
FC-HT			.63	.17	.14	-.25	.18	.12	-.20	.67	.29	-.16
PER-HT				-.26	.44	-.44	.11	-.05	-.41	.53	.17	-.07
C-HT					.02	.55	.59	-.11	-.12	-.05	.02	-.07
PER-WT						.38	-.05	-.39	-.27	.10	-.39	.05
C-WT							-.13	-.00	-.11	-.52	-.58	.04
P-ADH								-.34	-.09	.18	.40	.20
P-CO									.04	-.16	-.29	-.20
P-ETOH										-.43	-.32	-.02
C-ADH											.68	-.11
C-CO												.29

Table 3b. Correlation matrix of growth and physiological measures from soil tank tests.

------Periodic Waterlogging------					-----Continuous Waterlogging-----				
	TOTWT	ADH	CO	ETOH		TOTWT	ADH	CO	ETOH
HT	0.79	-0.01	-0.16	0.15	HT	0.78	-0.00	-0.06	-0.13
	0.00	0.87	0.29	0.27		0.00	1.00	0.65	0.30
	345	167	43	60		304	176	67	70
TOTWT		0.13	-0.09	0.04	TOTWT		-0.11	0.03	0.07
		0.10	0.59	0.78			0.15	0.81	0.59
		158	41	58			170	62	64
ADH			**	**	ADH			0.05	-0.34
								0.80	0.07
								30	30
CO				0.19	CO				0.04
				0.23					0.75
				43					67

* Abbreviations: YJ - Yellow Jacket Road test; HI - Horse Island Road test; FC - Farewell Corner test; PER - periodically waterlogged tanks; C - continuously waterlogged tanks; HT - height; WT - total weight; ADH - ADH activity; CO - anaerobic CO_2 production; ETOH - anaerobic ethanol production;

** Not measured on the same trees.

INDUCTION OF NEW HOST-CODED PROTEINS IN PINUS ELLIOTTII SEEDLINGS IN RESPONSE TO PATHOGEN AND WATER STRESS

J.V. Valluri [1/], E.J. Soltes [2/], R.J. Newton [3/] and B.G. Cobb [4/]

Abstract.–A 55 kD stress protein was induced in tissue culture grown slash pine seedlings infected with conidial suspensions of pitch canker fungus. Induction was evident in both hypocotyl and root sections of the seedlings after a lag phase of 16 hours. The stress protein is best extracted from stressed tissues with slightly alkaline Tris-HCl (pH 8.65) buffer. We have also investigated protein synthesis in water-stressed slash pine seedlings. Mannitol-induced water stress inhibited overall protein synthesis, with induction of unique stress proteins in the hypocotyl tissue. At -1.8 MPa a 46 kD stress protein was induced. At -2.5 MPa at least five stress proteins 82 kD, 71 kD, 70 kD, 64 kD and 58 kD were detected by SDS-PAGE and Fluorography.

Additional key words: *Pinus elliottii*, Hypocotyl, Stress protein, *Fusarium moniliforme* var. *subglutinans*, Water stress, SDS-PAGE, Protein induction.

The resistance of pine trees to pathogen and water stress is of large economic importance. The development of techniques to screen new germlines for stress tolerance has significant importance towards better understanding of the biochemistry of stress in trees as well as the development of more resistant varieties. Higher plants under stress have evolved a variety of morphological and physiological mechanisms to cope with adverse biotic and abiotic stresses. The initial biochemical events which occur in plants exposed to pathogen and water stress appear to be quantitative and qualitative changes in protein metabolism. It has been proposed that the initiation of resistance or tolerance mechanisms is mediated by *de novo* gene activation resulting in the synthesis of new proteins which possibly have a role in plant protection against stresses (Antoniw and White 1983).

Pathogen stress: During the last few years, several groups have initiated studies on the response of plants to pathogen stress with emphasis on the analysis of gene expression (Van Loon 1985). Since genetic information is ultimately expressed in nucleic acids and proteins via the processes of transcription and translation, specific alterations in nucleic acid and protein metabolism are important aspects of pathogenesis. Transcriptional metabolism and regulation of pathogen stress induced proteins have not been studied in commercial woody plants, such as conifers.

[1/2/3/]Department of Forest Science, TAES, Texas A&M University System, College Station, Texas.

[4/]Department of Horticulture, TAES, Texas A&M University System, College Station, Texas.

We now present experimental evidence indicating *de novo* synthesis of a new protein in slash pine (*Pinus elliottii*) in response to stress induced by the pitch canker fungus (*Fusarium moniliforme* var.*subglutinans*). Pitch canker incited by this fungus is a serious disease of pine plantations and seed orchards in the southern United States. The destructive potential of pitch canker is sufficient to warrant increased attention to identify cellular components of the host which may have a role in disease resistance.

Water stress: Pines exposed to water stress environment encounter several basic problems: (1) a reduction in the water potential of the sorrounding medium; (2) the buildup and interference of toxic metabolites with the normal biochemical and physiological processes of the cells; and (3) required nutrition ions must still be obtained despite the predominance of other ions. In stressful environments, higher plants show a variety of morphological and biochemical modifications. When plants undergo frequent periods of drought their ability to synthesize proteins is reduced (Bewley et al. 1983). If drought is not too severe, protein synthesis can resume upon subsequent return of the plant to full hydration. As a stress response, the effect of drought stress appears analogous to the heat shock response in animals, plants and bacteria. The stress treatment mediates a repression of pre-existing (non-stress) protein synthesis while inducing transcription and translation of a small number stress proteins (Dasgupta and Bewley 1984).

METHODS

We have established a tissue culture system to study the changes in protein synthesis in slash pine in response to pitch canker fungal infection and mannitol-induced water stress. This *in vitro* system with *Pinus elliottii* seedlings grown from embryos is used to study the induction of stress proteins at various time periods.

Establishment of seedlings: Slash pine seed were supplied by the Texas Forest Service. Seeds were placed in 30% hydrogen peroxide for 30 min and then soaked in 1% H_2O_2 at room temperature for 4 days. They were then surface sterilized in 15% Chlorox for 15 minutes and rinsed three times with sterile water. Embryos were removed aseptically, placed in Murashige -Skoog (MS) medium in sterile culture tubes, and grown in a controlled environment chamber with temperature of 24°C and 16 hr photoperiod (Murashige and Skoog 1962).

Fungal inoculum preparation and inoculation: The procedures for preparation of macro - and microconidia of *F. moniliforme* were modified from Barrows-Broaddus and Dwinnel. The initial fungal isolate was recovered from *Pinus elliottii* var.*elliottii* in Florida and was donated by G.M. Blakeslee (School of Forest Resources, University of Florida, Gainesville). Fungal cultures were grown on a PDA medium for 10 days with a 12 hr photoperiod at 24°C. Conidia were washed from the culture plates with sterile deionized water, and the number of conidia were estimated with a hemacytometer and the concentration was adjusted to 10^6 ml^{-1} . Ten μl of the inoculum were placed directly

on the hypocotyl with wounding by hypodermic needle. Controls were treated identically with distilled water in lieu of the inoculum. The plant material was incubated for 2, 4, 8, and 16 hr in an environmental chamber.

Mannitol-Induced Water stress: In separate set of experiments mannitol was added to the culture medium to obtain varying water potentials. Addition of 10% and 15% mannitol resulted in water potential of -1.8 MPa and -2.5 MPa respectively. In our preliminary studies on ^{35}S-methionine incorporation into slash pine hypocotyls, we have eliminated the use of polyethylene glycol (PEG) as a suitable osmoticum for water stress. The PEG interferes with protein extraction and migration on gel electrophoresis. Mannitol (10% and 15%) have shown to elicit water stress symptoms and has no effect on migration of proteins on gel electrophoresis. A wescor (Logan, Utah) model HR33T hygrometer with model G52 thermocouple psychrometer chamber in the dew point mode was used to measure osmotic potential of the medium. Seedlings were water stressed for 2, 4, 8 and 16 hours.

In vivo labelling and extraction of proteins: At the end of each stress period the seedlings were excised aseptically and divided to cotyledons, hypocotyls, and root sections which were then incubated in 1 ml of a solution which contained 50μCi ml^{-1} of ^{35}S methionine (> 500 Ci/mol; ICN) for 2 hours. After labelling, the sections were removed and washed with a solution containing 1mM methionine. They were homogenized in ice-cold 50mM Tris-HCl buffer (pH 8.65), containing 20mM KCl, and 10mM $MgCl_2$. The slurry was centrifuged at 13,000g for 15 minutes, and the supernatant fraction was used for protein analysis. For measurement of protein synthesis, 200μl of the supernatant was precipitated with 1 ml of 20% (w/v) trichloroacetic acid overnight in the cold. Fifty μl of the supernatant fraction were counted for total incorporation in 3 ml of a toluene scintillation mixture. The remaining supernatant and the pellet were washed with 5% TCA through fiberglass filters (Gelman Sciences Inc, A/E 2.5 cm diameter). The proteins trapped on the filter paper were rinsed twice with 5 ml of 95% ethanol and acetone. The filters were dried at 60°C and the radioactivity on the filters was directly counted in 3 ml of a toluene scintillation mixture.

Polyacrylamide gel electrophoresis: Total protein was estimated by the Bradford method using a Dynatech MR 650 microtiter reader (Bradford 1976). Gels were loaded based on equal amount of TCA precipitable counts from control and stress tissues. SDS-PAGE was carried out on 12% polyacrylamide gels containing 0.1% SDS (Laemmli 1970). After electrophoresis gels were treated with EN^{3}HANCE (New England Nuclear) prior to drying. Fluorography was performed on dried gels using Kodak X-Omat AR film at -70°C.

RESULTS AND DISCUSSION

Sixteen hours after inoculation, a new protein band was detected in extracts of both hypocotyl and root tissue from stressed tissue, but not in the uninoculated control tissues (Fig 1). The protein found in stressed pine hypocotyl and root tissue is of relatively high molecular weight (55 kD).

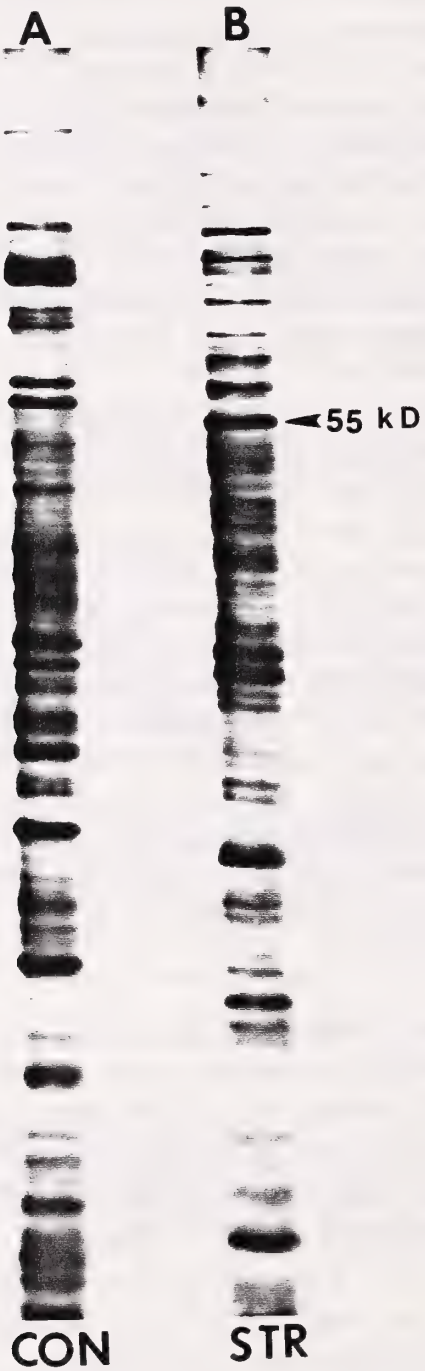


Figure 1. SDS-Polyacrylamide gel pattern showing induction of a 55 kD stress protein in response to pitch canker fungal infection (lane B). Lane A is the control tissue. Each lane contains equal amount of radioactive counts (2×10^5 cpm).

Relative mobilities were calculated for each of the standard proteins and for the stress protein. The apparent molecular weight of the stress protein was determined by matching the R_f value with the appropiate point on the standard curve. Analysis of 2, 4, and 8 hr stressed tissue did not reveal any synthesis of new proteins. Under these incubation times there was no fungal growth on the stressed tissue. In order to determine if the observed, high-molecular weight protein was a result of *de novo* synthesis in host tissue and not of fungal origin, native proteins in the fungal inoculum were analysed by SDS-PAGE, and stained with Bio-Rad's silver stain. No proteins were detected in the inoculum, suggesting that the new protein detected is of host origin and induced in response to pathogen stress. We hypothesize that the high molecular weight stress-protein induced in the pine hypocotyls is as a result of chemical mediation eliciting a signal for gene activation. Although the induction of stress protein was not detected until 16 hours after fungal challenge, Bradford assay showed increase in total protein in the stressed tissue, when compared to the uninoculated controls.

A striking feature of pathogen stress response in slash pine seedlings is that the stress protein synthesis occurs in addition to the normal protein synthesis. This is very different from the drought stress response of slash pine seedlings in which normal protein synthesis decreases so that only drought stress proteins are detectable on a gel. Both qualitative and quantitative changes were detected in protein patterns in slash pine hypocotyls subjected to -1.8 MPa and -2.5 MPa water stress for 24 hr. At -1.8 MPa slash pine hypocotyls responded to stress by induction of a 46 kD stress protein. At -2.5 MPa at least five stress proteins 82 kD, 71 kD, 70 kD, 64 kD and 58 kD were detected by SDS-PAGE and Fluorography (Fig 2). The synthesis of several proteins was reduced by stress, although that of others intensified. Stress recovery experiments were done by transferring the stressed tissue onto the control medium. Protein induction in tissue stressed at -1.8 MPa for 24 hr appears to recover, whereas in tissue stressed at -2.5 MPa the overall protein recovery does not occur. While new proteins were induced in stressed tissues, their capacity to conduct *in vivo* protein synthesis when the tissues are returned to control media is low. We regard such tissues as having acutely stressed.

Some studies have documented that the drought stress inhibited the incorporation of amino acids into proteins. The protein synthesis data obtained in our study reveal good incorporation of ^{35}S methionine label in the control tissue and a decreased incorporation in the stressed tissue. Water stress is a signal for the induction of new proteins, and we know at mild to moderate water stress the stress protein synthesis will eventually return to normal if that signal is removed. How the signal is percieved by the cell is a question that remains largely unanswered. We believe that under short term drought stress shock proteins are produced which are transient in nature and disappear when the stress is removed. However, in tissue stressed at -2.5 Mpa different pattern of protein synthesis occurs which includes strong induction of new proteins which are stable even after the stress is removed.

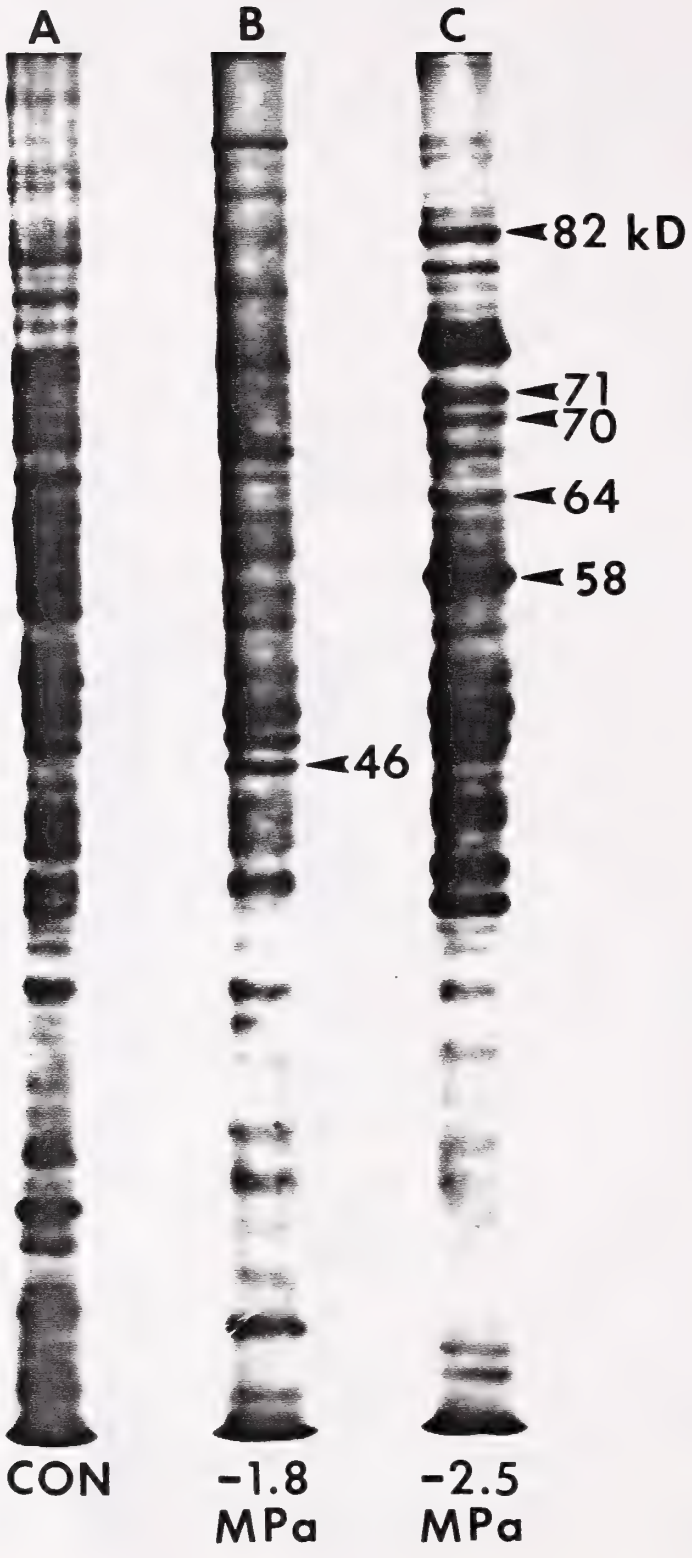


Figure 2. SDS-Polyacrylamide gel pattern showing induction of unique proteins in hypocotyl tissue in response to water-stress at -1.8 MPa (lane B) and -2.5 MPa (lane C). Lane A is the control tissue. Each lane contains equal amount of radioactive counts (2×10^6 cpm).

CONCLUSIONS

In general, stress proteins are absent in healthy growing plants. Induction of these proteins occurs in response to both biotic and abiotic stress conditions. While some researchers have suggested that the pathogen-stress proteins may have a role in induced systemic resistance, their function is still speculative. The evidence gathered thus far establishing a link between pathogen-stress proteins and induced resistance is comprehensive, but it is entirely correlative.

The resistance of pine trees to water stress has received much attention due its economic importance. Any metabolic adaptation to water stress involving a change in gene expression requires an alteration in protein and /or nucleic acids which may have a role in drought tolerance mechanisms. Cellular-level tolerance might be most amenable to genetic manipulation because few genes for metabolic processes are involved in osmoregulation. Additional advantage would include evaluating potential germplasm for drought tolerance, assuming that similarity exists between cellular level responses and whole-plant level responses under field conditions.

LITERATURE CITED

Antoniw, J.F. and R.F. White. 1983. Biochemical properties of the pathogenesis related proteins from tobacco. Netherlands Journal of Plant Pathology. 89:255- 264.

Barrows-Broaddus, J. and L.D. Dwinell. 1983. Histopathology of *Fusarium moniliforme* var. *subglutinans* in four species of southern pines. Phytopathology. 73:882-889.

Bewley, J.D., K.M. Larsen, J.E. Papp. 1983. Water stress induced changes in the pattern of protein synthesis in maize seedling mesocotyls. A comparison with the effects of heat shock. Journal of Experimental Botany 34:1126-1133.

Bradford , M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254.

Dasgupta, J. and J.D. Bewley. 1984. Variations in protein synthesis in different regions of greening leaves of barley seedlings and effects of imposed water stress. Journal of Experimental Botany 35:1450-1459.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Murashige, T. and D.F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 1:473-497.

Van Loon, L.C. 1985. Pathogenesis-related proteins. Plant Molecular Biology 4:111-116.

SEED ORCHARD MANAGEMENT I

MODERATED BY DR. J. R. TOLIVER

Louisiana State University

SEED ORCHARD MANAGEMENT: SOMETHING OLD AND SOMETHING NEW

J. B. Jett[1/]

Abstract.--Today, seed orchard management in the southeastern United States stands at a crossroad. Thirty-four years of research and experience have brought regional seed orchard production to the point that large surpluses of genetically improved seeds are available for sale. Despite this progress, unanswered questions remain regarding management techniques for advanced generation seed orchards. Older orchards producing surplus seed present one set of problems. Young second generation seed orchards and the third generation seed orchards planned for the turn of the century will require cost-effective management to promote rapid orchard development and early seed production which is a different set of challenges.

Continuing research on irrigation, subsoiling, fertilization, supplemental mass pollination and roguing practices is needed to provide improved understanding of these management practices. Additional research is required if we are to understand the role of seed orchard ground covers, provide better fertilization prescriptions, improve pollen handling procedures, understand the influence of selected rootstock, and understand intensive levels of orchard roguing.

INTRODUCTION

To sustain and increase forest productivity in the southeastern United States, great emphasis was placed on the genetic improvement of forest trees approximately thirty years ago. Seed production areas and seed orchards were hurriedly established to supply genetically improved seed for the vast regeneration needs. Approaches to orchard design and techniques for establishing and managing the seed producing units were widely debated and were almost as diverse as the various organizations initiating the work.

Research and experience over the past thirty years has brought seed orchard production in the southeastern United States to the point of wide scale surplus production of first generation seed. Yet, despite the seed surpluses of slash, loblolly, and Virginia pine (*Pinus elliotti*, *P. taeda*, and

---

1/ Associate Director, North Carolina State University-Industry Cooperative Tree Improvement Program and Associate Professor, School of Forest Resources, Raleigh, North Carolina.

Paper No. 11094 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina 27695. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

P. virginiana), there is, more than ever, an acute need for seed orchard management research. Three factors drive this continuing need for seed orchard management research: (1) Seed production from second generation seed orchards is not sufficient to meet regeneration requirements; (2) third generation seed orchards will be a reality by the mid-1990's; and, (3) the forest industry is under the grip of management driven by the need for short term economic gain. All of these factors contribute to the pressure to produce more seed sooner and more economically.

Today, seed orchard management is at a crossroad between generations. Very mature orchards and very young orchards exist side by side requiring different approaches. Mature orchards require inputs to maintain seed quality and strategies designed to deal with surplus seed. New orchards require focusing on factors to promote growth and initiate seed production.

It is the purpose of this paper to take a brief look at some areas of seed orchard management that are being researched, provide some new, as yet unpublished, research results and to pose questions for future research. However, it is impossible to address all aspects of seed orchard operations. As an example, seed orchard insect control practices and strategies continue to require a great deal of research. The whole area of rootstocks and rootstock/scion interactions and how they affect graft vigor and fecundity offers an exciting area of research. However, these topics and others were deliberately omitted from this discussion because either research results are not currently available or the author believes the topics could be better addressed in separate papers.

SEED ORCHARD MANAGEMENT TECHNIQUES

Seed Orchard Site Selection

Economists have determined that forest tree seed orchards are sound investments only when they are fully productive (Talbert et al. 1985). Perhaps the single most important factor in promoting a fully productive seed orchard is seed orchard site selection. Orchard productivity is dependent upon climatic, edaphic, management and genetic factors. Three of these factors are site related and underscore the need to carefully choose seed orchard sites.

While it is not necessary to locate an orchard in the native provenance of the seed source, an excellent rule of thumb is under no circumstances should an orchard be placed in a more harsh environment than that from which the ortets were selected. Moving loblolly pine seed orchards south has been conducive to seed production (Schmidtling 1978, 1983; Richmond and McKinley 1986). Within the North Carolina State University-Industry Cooperative, a trend is to move northern orchard operations to south-central Georgia. Experience has shown that orchards in this region produce more seed per year and reach commercial production sooner than in northern locations.

Despite the positive influence on seed production, important questions are being raised about how this type of movement might influence cold hardiness of orchard progenies through possible local nonorchard pollen contamination. Other questions concern parental environment after-effects on progenies that extend beyond the immediate questions of seed size and vigor.

Irrigation

Irrigation of seed orchards has received much interest over the years from both researchers and orchard managers. Neither published reports nor experience provide a clear understanding of the impact of irrigation on cone production. Some proponents of orchard irrigation believe that irrigation of young seed orchards is worthwhile because of enhanced tree growth. The larger trees should ultimately result in larger cone crops via enhanced crown size. An irrigation-nitrogen fertilization study in a young second generation loblolly pine orchard documented the impact of irrigation on tree crown size. Crown volume of irrigated trees was found to be 32 percent greater than non-irrigated trees (Jett 1983). Importantly, irrigated trees averaged two more potential flowering sites per primary branch than did nonirrigated trees. Although full growing season irrigation was stopped in this orchard at the end of the 1983 growing season, measurements taken following the 1986 growing season (age seven) revealed that the early size superiority of d.b.h. and crown volume for the irrigated trees was being maintained (table 1). While the differences are not statistically significant, irrigated trees averaged 19 more cones per tree than nonirrigated trees during the 1986 cone harvest (table 1).

A continuing irrigation timing study installed in 1978-79 in a four year-old 1.5 generation loblolly pine seed orchard located in the northern piedmont of South Carolina is yielding erratic information. After one year of treatment, trees irrigated mid-June through July produced a significantly greater flower crop than did other treatments (Harcharik 1983). Subsequent analyses indicate that both male and female strobilus production is erratic by treatment and year (table 2). Subsequent analyses of cones/tree harvested in 1985 and 1986 failed to reveal any significant treatment differences.

Irrigation studies need to be long term in order to truly gauge treatment effects over highly variable year effects. A real need is to learn how to account for natural precipitation effects which can obscure irrigation effects. Understanding how irrigation can influence seed production depends on a better understanding of how irrigation effects basic tree physiology and nutrition. Other, more applied questions, need to focus on the potential cone and seed quality effects.

Subsoiling

Although subsoiling has been used by American agriculture for over 50 years to alleviate soil compaction (Cassel 1979), it was first used in a loblolly pine seed orchard in 1964 to alleviate what was considered to be adverse soil compaction (Gregory 1975). The positive response of this early subsoiling effort resulted in increasingly wide use of this cultural practice.

At present, we do not clearly understand the reasons behind the positive response to subsoiling. Flowering responses might be due to stresses induced by root pruning as suggested by Gregory and Davey (1977), or subsoiling could result in improved tree vigor and higher seed production through amelioration of soil conditions. Root pruning may represent an immediate short-term response to subsoiling which soon disappears as the roots quickly recover and expand beyond the subsoil trench. Experience has shown that the roots branch prolifically at the point where they were severed by the subsoiler. On the

Table 1.--Main treatment effect means for total height, d.b.h. and crown volume[1/] following the 1986 growing season and for cones per tree from the 1986 harvest for an irrigation-fertilization study in a seven-year-old second generation loblolly seed orchard

Variable	Irrigation	No Irrigation	
Total Height (m)	8.5	8.3	NS [2/]
D.b.h. (cm)	21.9	19.5	LSD.05 = .96
Crown Volume (m^3)	75.6	55.2	LSD.05 = 9.7
Cones/Tree	159	140	NS

[1/] Crown volume calculated on the basis of a cone using mean crown diameter and live crown length
[2/] NS = statistically nonsignificant at $P \leq .05$

Table 2.--Male and female strobili production in loblolly pine as affected by irrigation timing, 1982-1984

	Male Strobili				Female Strobili			
Treatment[1/]	1982	1983	1984	Mean	1982	1983	1984	Mean
	------ Clusters/tree[2/]-------				------Strobili/tree[2/]----------			
DD	2.7 a	8.7 a	24.7 b	12.0	17.7 a	40.3 a	50.7 a	36.2
DW	1.7 a	10.4 a	44.2 ab	18.7	12.5 a	40.1 a	41.6 a	31.4
WD	1.8 a	10.9 a	41.8 ab	18.2	12.3 a	50.5 a	42.0 a	35.1
WW	3.7 a	16.8 a	84.4 a	34.9	12.7 a	46.1 a	37.9 a	32.2

[1/] DD = no irrigation
WW = irrigation throughout the growing season
DW = no irrigation 16 June - 31 July (with irrigation rest of the season), and
WD = no irrigation 1 August - 15 September
[2/] Means with a common letter are not significantly different at the .05 level

other hand, alterations to the soil structure represent a long term influence benefiting the tree through improved health and vigor. Both long- and short-term benefits to subsoiling were noted by Schmidtling (1986) in Virginia pine.

Accumulating evidence from a North Carolina State University Cooperative study to evaluate the frequency and intensity of subsoiling lends support to the idea that the positive response to subsoiling consists of more than stress responses. Fifth year study results in a 11 year-old orchard following the 1985 growing season indicate that none of the treatments significantly influenced (neither positively nor negatively) total tree height, rate of growth, d.b.h. or crown size. In contrast to the lack of response in tree growth variables, there was a significant response to treatments on the 1984 cone crop (table 3). Despite the lack of statistically significant treatment effects in 1985 and 1986, there was a trend for the four subsoiling treatments to promote the number of cones per tree compared to the control.

Some very recent and still preliminary results from this subsoiling study indicate that the subsoiling treatments increased the number of seeds per cone in comparison to the nonsubsoiled control. There were no significant differences between treatments in the number of large, medium or small sized seed.

Table 3.--Mean number of cones per tree by year following five subsoiling frequency and intensity treatments in a loblolly pine seed orchard

Treatment [1/]	1984 [3/]	1985 [4/]	1986
	Cones/Tree		
Multiple rip - 3 year cycle	144a	117a	73a
Multiple rip - 2 year cycle	140a	174a	93a
Single rip - 3 year cycle [2/]	117ab	139a	74a
Single rip - 2 year cycle	108 b	152a	73a
Control - no subsoiling	97 b	128a	58a

1/ Multiple ripping involves 3 parallel cuts on opposite sides of a tree. A three year cycle entails ripping every other year as opposed to every year as in the 2 year cycle

2/ Equivalent to operational subsoiling

3/ Means followed by the same letter are not significantly different at the 5 percent level

4/ This harvest follows an operational orchard roguing which involved removing 33 percent of study trees

To date, research on subsoiling has largely focused on gross cone production and tree growth parameters. A necessary step in fine tuning this practice will be to understand how subsoiling alters plant nutrition, moisture relationships and hormonal balances. How transient or, conversely, longlasting the affects might be needs much more research effort.

Orchard Fertilization

The most widely accepted cultural practice to enhance cone production in southern pine orchards is fertilization. While the mechanism through which nitrogen enhances flowering in conifers remains unclear (Ross and Pharis 1985), its use in conifers has been very effective (Owens and Blake 1984; Schmidtling 1975).

The most pressing need to refine seed orchard fertilization as a management tool is a better means of detecting nutritional needs. While much remains to be learned about foliar nutrient levels and how they relate to flower production, foliar analyses offer a sensitive diagnostic and predictive tool to supplement soil analyses for making seed orchard fertilization recommendations. Recent research by the North Carolina State Cooperative Tree Improvement Program and the North Carolina State University Forest Nutrition Cooperative has been directed at developing guidelines for the use of foliar nutrient analyses to prescribe seed orchard fertilization. Preliminary results involving bimonthly collection of foliage samples from numerous clones for 12 months in one piedmont and one coastal plain loblolly pine seed orchard provide some basic understanding of sampling requirements. As anticipated, there were significant clonal differences for essentially all elements evaluated (N, P, K, Ca, and Mg). There was a significant clone x sample date interaction, and the sampling date was a significant source of variation for foliar nitrogen at both locations. Both sites displayed a similar nitrogen concentration x date response, but the two sites have markedly different nitrogen concentrations at almost every point in time. Foliar levels peaked at the coastal plain and piedmont locations at the May sampling date and dropped rapidly as the female flower initiation for loblolly pine period of mid-July to August approached. If elevated levels of foliar nitrogen are conducive to flower stimulation, these results suggest June is the best time to guide summer nitrogenous fertilizer applications.

A potentially useful approach to interpreting foliar nutrient analyses data is through use of the Diagnosis and Recommendation Intergrated System (DRIS) (Beaufils 1973). This technique has been successfully employed in several agricultural crops to overcome problems of foliar diagnosis and interpretation. The DRIS approach considers all nutrients together and can indicate both the limiting nutrients, and also which ones are likely to become limiting (Davee et al. 1986). Currently, the North Carolina State University Cooperative is initiating work to apply the DRIS procedure to seed orchard management.

Pollen Management

Seed orchard pollen management has experienced renewed interest in the last few years. Early concern about pollen management centered on attempts to minimize pollen contamination from sources outside the orchard and to increase

both the quantity and genetic quality of the seed. Many studies (e.g. Wright 1953; McElwee 1960, 1970; and Silen 1962) evaluated pollen dispersion and developed strategies to minimize pollen contamination in seed orchards such as the pollen dilution zone, size and arrangement of orchards and orchard site selection.

The movement of northern seed orchards south raised hopes that it might be possible to obtain pollen isolation by having the flowering of the orchard clones out-of-phase with the local populations. Experience to date has been mixed with some orchard managers reporting asynchronous flowering and others unable to detect any differences. Some orchards with minimal internal pollen production have still produced many viable seeds. As orchards mature, the significance of any partially out-of-phase local pollen may be reduced. Data from an experimental loblolly pine orchard in south Texas, well out of the loblolly pine range, seem to indicate the presence of little, if any, contaminating foreign pollen (Richmond and McKinley 1986).

Published reports indicate that pollen contamination in seed orchards varies widely: Greenwood and Rucker (1985) reported in a study of four orchards that contamination varied from 31 to 88%. Friedman and Adams (1981), using electrophoretic techniques, estimated contamination of 28%. Economic analyses of the consequences of pollen contamination indicate that appropriate corrective measures depend upon the number of acres an organization will be planting, the amount of pollen contamination and the gene frequency diffential for desired characteristics between the orchards and the source of pollen contamination (Sniezko 1981). As tree improvement programs develop advanced generation seed orchards, the economic value associated with reducing pollen contamination increases significantly. As previously noted, selecting seed orchard sites for the next cycle of seed orchards may require re-evaluation of the trend toward moving northern seed orchards south. Obviously, important questions remain to be answered about the significance of pollen contamination over a variety of locations and how to minimize the impact of contamination.

Supplemental mass pollination (SMP) procedures have received attention as a means to reduce the impact of self pollination in first generation production orchards and to increase the supply of desirable pollen in young seed orchards where pollen is often lacking. In recent years, SMP has been evaluated as a method to improve genetic gains through full-sib crosses and, to some extent, to produce interspecific hybrids. Supplemental mass pollination procedures have proven to be quite effective in improving seed yields of Pinus spp. in South Africa (van der Sijde 1969, and Denison 1973). Although the feasibility of SMP has been demonstrated on an experimental basis, much developmental work under operational conditions remains to be done (Bridgwater and Williams 1983). To date, relatively few organizations have attempted to use SMP on an operational basis. However, SMP is likely to become an important tool for many seed orchard managers.

Orchard Roguing

Currently, the profitability and realization of genetic gain pivots on the production of seed for use in regeneration programs. With the exception of supplemental mass pollination, orchard roguing is the only orchard manage-

ment technique which can increase the genetic quality of the seed produced and, at the same time, frequently increase seed production per tree.

Cone production is definitely affected by the amount of light the tree crown receives. Full crown release in a loblolly pine plantation resulted in a four-fold increase in cone production during the fourth year following crown release (Bilan 1960). Similar increases have been observed in thinned and rogued seed orchards across the southeastern United States. In fact, orchard thinning and roguing based on genetic test information have become established practices throughout the life of first generation seed orchards and is now being routinely applied to second generation orchards.

Seed production by many organizations across the south has been so successful in recent years that an over supply of genetically improved loblolly pine seed has resulted. For example, during the 1986 cone harvest season, an estimated 8,182 kilograms of loblolly pine seed were deliberately not harvested by members of the North Carolina State University Cooperative Program. However, there has never been an over supply of the very best genetically improved seed. To expand production of very high genetic quality seed, more and more organizations are turning to very intensive seed orchard roguing. This type of roguing leaves only the best nine to 12 parents. The genetic quality can be increased four to five percent, and operating costs are substantially reduced.

Two concerns with roguing to these low stocking levels are the potential increase in the amount of self-pollination and the potential for greater contamination levels by nonorchard pollen. Preliminary research findings from orchard blocks rogued to four and 12 clones (17 and 54 ramets/hectare, respectively) have indicated equal outcrossing rates. If the remaining clones are good pollen producers or, if supplemental mass pollination was employed, selfing and background pollen contamination might be of reduced concern. Very intensive roguing should be viewed as experimental, pending further studies.

Orchard Ground Cover Management

Maintenance of a well-established ground cover in southern pine seed orchards has been a standard practice providing erosion control, enhancing trafficability, and reducing compaction due to vehicular traffic. However, little attention has been given to the choice of ground cover, how various ground covers might interact with the orchard trees, and how they might affect orchard management costs.

In horticultural crops, there are numerous cases where the grass cover crop competed directly with the trees, influencing both growth and fruit production (Haynes 1980, Skroch and Shribbs 1986). Various cover crop species compete directly for moisture and nutrients, particularly nitrogen (Proebsting 1958, Fales and Wakefield 1981, and Jordon 1982). How serious grass competition for N might be is unknown, especially in view of the high levels (80 kg/ha/yr) of N commonly applied to seed orchards. In the case of a cool season grass, like tall fescue (Festuca arundinacea), N competition may be mild during the summer but intense during the cooler portions of the year.

Grasses also have an influence on the use of soil systemic insecticides such as Furadan®. Sod effectively competes with trees for soil systemics, necessitating application of larger amounts of insecticides. However, sod helps to prevent runoff of insecticides, thus protecting water resources.

Horticultural literature contains numerous references dealing with allelopathic relationships between various ground covers and weeds and tree crops (see Putnam 1983 and 1986). However, the presence or absence of allelopathic relationships and their possible importance in southern pine seed orchards are unknowns.

Periodic mowing continues to be the most commonly employed method of controlling orchard turf. Recent estimates of costs per hectare per cut range from $27 to $37 (Jett and Weir 1984). With three to six mowings per year, mowing is expensive. Additionally, the costs of mowing are not limited to the obvious direct costs of personnel and equipment but extend to the indirect costs associated with soil compaction, especially when operating equipment during periods of high soil moisture. Careless mowing also results in damage to the trees.

Strip spraying of Roundup®, Oust®, and even Velpar® along the tree rows in older orchards has become commonplace. This practice essentially eliminates the need to cross-mow in the orchard and results in at least three advantages: 1) Equipment traffic in the orchard is significantly reduced; 2) mower and tractor damage to the trees is essentially eliminated; and 3) strip spraying results in substantial savings on mowing costs. Various orchard managers have calculated savings of approximately 12.5 to 25 percent, more than enough to offset herbicide spraying costs.

Increasingly, orchard managers are asking questions about reducing costs beyond those achieved through strip spraying herbicides. What are the possibilities of using grass species requiring fewer cultural inputs, or using chemical growth retardants and sub-lethal rates of herbicides to reduce turf management costs and expedite operations like cone harvest? Understanding how various species of ground covers or orchard floor management schemes might affect moisture availability, nutrient status, tree growth and flowering in southern pine seed orchards awaits research.

CONCLUSIONS

Opportunities abound for research into virtually every facet of seed orchard management. At this point in time, the research needs focus on a more basic understanding of how various cultural practices impact tree development and flowering. This more basic understanding should allow refinement of management procedures, while at the same time offering a cost savings to orchard programs.

Probably some of the most pressing research should address the movement of northern source seed orchards to southern locations. If this type of movement can be done without jeopardizing cold hardiness, the benefits to northern tree improvement programs are important. Additionally, understanding pollen contamination and how to effectively deal with it are of real importance to tree improvement programs.

LITERATURE CITED

Beaufils, E. R. 1973. Diagnosis and recommendation integrated system (DRIS). Soil Sci. Bul. No. 1. Univ. Natal, S.A. 131 p.

Bilan, M. V. 1960. Stimulation of cone and seed production in pole-sized loblolly pine. For. Sci. 6:207-220.

Bridgwater, F. E. and C. G. Williams. 1983. Feasibility of supplemental mass pollination to increase genetic gains from seed orchards. Proc. 17th So. For. Tree Imp. Conf., Athens, GA. pp. 78-88.

Cassel, D. K. 1979. Subsoiling. Crops and Soils. 32(1):7-10.

Davee, D. E., T. L. Righetti, E. Fallahi, and S. Robbins. 1986. An evaluation of the DRIS approach for identifying mineral limitations on yield in 'Napolean' sweet cherry. J. Amer. Soc. Hort. Sci. 111:988-993.

Denison, N. P. 1973. A review of aspects of tree breeding in the Republic of South Africa with special reference to the Eastern Transvaal. In J. Burley and D. G. Nikles (ed.). Selection and breeding to improve some tropical conifers. IUFRO, Gainesville, Fla., Vol. 2, p. 1-8.

Fales, S. L. and R. C. Wakefield. 1982. Effects of turfgrasses on the establishment of woody plants. Agron. J. 73:605-610.

Friedman, S. T. and W. T. Adams. 1981. Genetic efficiency in loblolly pine seed orchards. Proc. 16th So. For. Tree Imp. Conf., Blacksburg, Va. pp. 213-224.

Gregory, J. D. and C. B. Davey. 1977. Subsoiling to stimulate flowering and cone production in a loblolly pine seed orchard. South. J. Appl. For. 1(2):20-23.

Gregory, J. D. 1975. Subsoiling to stimulate flowering and cone production and ameliorate soil conditions in loblolly pine (Pinus taeda L.) seed orchards. Ph.D. Diss., N. C. State University, Raleigh. 121 p.

Greenwood, M. S. and R. C. Schmidtling. 1981. Regulation of catkin production. In E. C. Franklin (ed.), Pollen Management Handbook, Chapter 4. U.S.D.A. Forest Service, Ag. Hdbk. 587, pp. 41-58.

Haynes, R. J. 1980. Influence of soil management practice on the orchard agro-ecosystem. Agro-Ecosystems 6(1):3-32.

Harcharik, D. A. 1983. The timing and economics of irrigation in loblolly pine seed orchards. Ph.D. Diss., N. C. State University, Raleigh. 138 p.

Jett, J. B. and R. J. Weir. 1984. Integrated pest management in seed orchards--vegetation. Integrated Pest Management Symposium. Athens, Ga. June 18-21, 1984.

Jett, J. B. 1983. The impact of irrigation and supplemental nitrogen fertilization on the development of a young loblolly pine seed orchard. Ph.D. Diss., N. C. State Univ., Raleigh. 42 p.

Jordon, L. 1982. Management of cover vegetation in citrus orchards. In J. C. Miller (ed.). Workshop Proceedings Cover Crop Production, Cover Crops and Sods as Living Mulches. Corvallis, OR. Oregon State Univ. and the International Plant Protection Center. p. 73-79.

McElwee, R. L. 1968. An analysis of factors contributing to the flight patterns of loblolly pine pollen. M. S. Thesis, N. C. State Univ., Raleigh, N. C.

_____. 1970. Radioactive tracer techniques for pine pollen flight studies and an analysis of short-range pollen behavior. Ph.D. Thesis, N. C. State Univ., Raleigh, N. C. 97 p.

Proebsting, E. L. 1958. Yield, growth and date of maturity of Elberta peaches as influenced by soil management systems. Proc. Amer. Soc. Hort. Sci. 72:92-101.

Putnam, A. R. 1983. Allelopathic chemicals. Chem. and Engineering News 144:34-45.

_____. 1986. Allelopathy: Can it be managed to benefit horticulture? HortSci. 21:411-413.

Richmond, G. B. and C. R. McKinley. 1986. An experimental seed orchard in south Texas. Jour. For. 84(7):19-20.

Ross, S. D. and R. P. Pharis. 1985. Promotion of flowering in tree crops: different mechanisms and techniques, with special reference to conifers. In M.G.R. Cannell and J. E. Jackson (ed.). Attributes of Trees as Crop Plants, Institute of Terrestrial Ecology, England. p. 383-397.

Schmidtling, R. C. 1975. Fertilizer timing and formulation affect flowering in a loblolly pine seed orchard. Proc. 13th So. Forest Tree Imp. Conf., Raleigh, N. C. p. 153-160.

_____. 1978. Southern loblolly pine seed orchard produce more cone and seed than do nothern orchards. In F. Bonner (ed.). Proc.: A Symposium on Flowering and Seed Development in Trees. U. S. Forest Service and IUFRO. Starkville, Miss. p. 177-186.

_____. 1983. Geographic location affects flowering of loblolly pine. Proc. 17th So. For. Tree Imp. Conf.. Univ. of Georgia, Athens, Ga. p. 42-48.

_____. 1986. Long-term effects of subsoiling on growth and flowering in a Virginia pine seed orchard. Proc. 9th North American Forest Biology Workshop. Stillwater, OK. (in press).

Silen, R. R. 1962. Pollen dispersal considerations for Douglas-fir. Jour. For. 60:790-795.

Skroch, W. A. and J. M. Shribbs. 1986. Orchard floor management: an overview. HortSci. 21:390-394.

Sniezko, R. A. 1981. Genetic and economic consequences of pollen contamination in seed orchards. Proc. 16th So. For. Tree Imp. Conf., Blacksburg, Va.. pp. 225-233.

Talbert, J. T., R. J. Weir, and R. D. Arnold. 1985. Costs and benefits of a mature first-generation loblolly pine tree improvement program. Jour. For. 83:162-166.

van der Sijde, H. A. 1969, Bending trees as a standard practice in pine seed orchard management in South Africa. FAO, IUFRO, 2nd World Consult. on Forest Tree Breeding, Washington, D. C. p. 1376-1379.

Wright, J. W. 1953. Pollen-dispersion studies: some practical applications. Jour. For. 51:114-118.

Value of production orchards based on two cycles of breeding and testing

Claire G. Williams and J. E. de Steiguer 1/

Abstract.--The net present values of two orchard alternatives were compared: 1) a production orchard per breeding-testing cycle (one-cycle orchard) and 2) a production orchard after two cycles of breeding and testing (two-cycle orchard). The specific question in current breeding programs is whether to install the third generation production orchard (one-cycle strategy) or delay orchard establishment until another breeding cycle can be completed (two-cycle orchard). Delaying orchard establishment and extending the service life of the second generation seed orchard (two-cycle orchard) was profitable if gains from progressive re-selection (roguing plus use of family-specific information) were 3.0 to 5.0%. The one-cycle orchard strategy was better if gains from orchard roguing were less than 5% and additional use of family-specific data had no value.

Additional keywords: Loblolly pine, one- and two-cycle production seed orchards, third generation orchard establishment.

There have two been classic alternatives to use of early selection technology. One is to establish a seed orchard after each shortened breeding cycle. The other is to delay orchard establishment until two or more breeding cycles have been completed. It has been suggested that it will not be possible to install seed orchards at very short generation intervals (van Buijtenen, 1981). The two-cycle production orchard may be profitable particularly if later years of an orchard's service life are highly valued.

The specific question as it pertains to the current cycle of loblolly pine tree improvement is as follows: should a third generation orchard be established or should establishment of the next orchard be delayed until one more cycle of accelerated breeding has been completed?

1/ Visiting Assistant Professor, School of Forestry and Environmental Studies, Duke University 27706 and Project Leader, Forest Economics, USDA U.S. Forest Service, Research Triangle Park, N.C. 27709, respectively.

BACKGROUND

The relative merit of the establishment of a third generation orchard and the alternative of using second generation seed longer is determined by service life of the third generation orchard and the value of seed from the added years of second generation orchards.

The third generation orchard's revenue depends on its seed's genetic value and its total production. The orchard's seed production is determined primarily by the length of time before its successor fulfills regeneration needs (orchard service life). The service life can be reduced to the point that the orchard is obsolete before it can yield an acceptable return on the establishment investment. If so, the two-cycle orchard is likely to more profitable.

The two-cycle strategy also has appeal because the seed from the later years of the orchard service life has greater genetic value than seed from the earlier years. Several years after a seed orchard reaches commercial harvest, information becomes available on the orchard clones' offspring (family) performance. This information may be used only to genetically rogue the orchard but it may be used to make additional production population gains.

Gains from additional re-selection (after roguing) are readily obtained. Cone harvest can be limited to the better maternal parents. Commercial-scale supplemental mass pollination or controlled-pollination can increase gains through re-selection on the maternal parents or through the exploitation of specific combining ability. Matching families to sites or the anticipated product demand may also boost gain (Gladstone, 1975; Duzan et al., in review). The additional use of family-specific information has a monetary value specific to each tree breeding organization. In general, the longer an orchard's service life, the greater the opportunity to identify and exploit these family-specific characteristics.

Our objective was to determine how high gains from re-selection within the second-generation production population must be to make the two-cycle orchard strategy at least as profitable as the one-cycle orchard strategy.

METHODS

The service life of the third generation orchard was determined by the generation interval which will hypothetically occur after the establishment of the third

generation orchard. The generation interval was set at 20 years through a) use of selection for the fourth generation orchard at four years (rather than selection at six years) and b) the assumption that the fourth generation orchard reaches commercial harvest after six years rather than after eight years (e.g. Jett, 1983) (Table 1).

Table 1. Hypothetical generation interval after establishment of the third generation orchard. The service life of the orchard was 11 years.

Activity	Years	References
Breeding	5	Greenwood et al (1984)
Tests established over two consecutive years	1	Talbert et al (1981)
Selection	4	
Time from selection to outplant grafts	1	Byram et al (1986)
Time from outplant to commercial harvest	6	
Time required for orchard to fully meet demand	3	
Total	20	

The criterion for comparing the one- and two-cycle orchards was difference in net present value. We have used a marginal analysis where all costs identical to the mutually exclusive alternatives were eliminated. All costs and revenues were reported in constant 1987 dollars. We have assumed the third generation orchard would be installed on 1987 technology although projected establishment is for the mid- 1990's (McKeand and Bridgwater, 1986). Only its service life will be altered by the changes in accelerated breeding technology and orchard development.

Assumptions

1. Third-generation seed yield projections

   Each orchard was sized to meet the regeneration demands four years after the onset of flowering because the genetic quality improves in young orchards as more clones contribute pollen (Zobel and Talbert, 1983, page 443). The seed to seedling ratio was 1 lb. to 8000 (van Buijtenen and Saitta, 1971). Ten acres was added for roads, irrigation and storage. Orchard production was 50 lb. per acre at full production (Bey et al. 1986).

2. Establishment costs

Establishment costs used by van Buijtenen and Saitta (1971) were converted to 1987 dollars with the producer price index (Anonymous, 1987). Agricultural-quality land costs were estimated to be $1350.00 per acre assuming a 4% annual increase since 1979 (de Steiguer, 1982). A real discount rate (i.e. net after inflation) of 8% was used. Orchard establishment costs were considered fixed or variable as follows:

Fixed costs:	$54,520.00
Road construction	
Equipment storage	
Equipment purchase	
Irrigation system	
Variable costs for acres in production:	$ 749.33/acre
Survey and site preparation	
Rootstock	
Grafting and outplant	

Harvest costs have been deleted since harvest in older orchards is not necessarily more costly for all orchard mangement regimes (Ed Sossaman, Weyerhaeuser Company, pers. comm.). We assumed that the orchard would be installed adjacent to an existing orchard facility but that additional land purchase was required. The cost of a 26-acre orchard for an average forestry organization was conservatively estimated to be $101,609.

3. Prediction of genetic gains

Percentage genetic gains from breeding and testing were assumed to increase linearly across the second and third generation orchards. Genetic gains per cycle (G) for the second generation orchard were 4.4% and doubled for the third generation. Gain per cycle for the third generation was expressed as incremental over second generation gains from breeding and testing (i.e. an additional 4.4%). Gain was estimated for selection of top full-sib crosses plus the best individual within each of the selected crosses. The gain estimates for 24-clone orchards were based on a breeding region in the North Carolina State University-Industry Cooperative (McKeand and Bridgwater, 1986; Talbert et al. 1981). The prediction equation was as follows:

$$G = i_f \, Cov_{A,P} / \sigma_{\bar{p}_j^+} \quad i_i \, Cov_{A,P} / \sigma_{p_j} \qquad \text{(Equation 1)}$$

i_f = selection intensity for families
= 1.76 (24 out of 250 unrelated crosses)

i_i = selection intensity for individuals
= 2.62 (1 out of 144 full-sibs)

$Cov_{A,\bar{P}}$ = Covariance between breeding values of selection goal at age m and the family means of the orchard candidates at age j.
= 1/4 Cov

$Cov_{A,P}$ = Covariance between breeding values of selection goal at age m and the phenotypes of the orchard candidates at age m.
= 1/2 Cov

$\sigma_{\bar{P}_j}, \sigma_{P_j}$ = Phenotypic standard deviations of the family mean and the candidates, respectively. Dominance variance was set to zero.

$$\sigma_{\bar{P}_j} = \left\{1/2\sigma_a^2 + 1/4\sigma_d^2 + 1/rn(1/2\sigma_a^2 + 3/4\sigma_d^2 + r\sigma_{rf}^2 + \sigma_e^2)\right\}^{1/2}$$

$$\sigma_{P_j} = \left\{1/2\sigma_a^2 + 1/4\sigma_d^2 + 1/2\sigma_a^2 + 3/4\sigma_d^2 + \sigma_{rf}^2 + \sigma_e^2\right\}^{1/2}$$

where σ_a^2 = additive genetic variance
σ_d^2 = dominance variance
σ_{rf}^2 = block by family interaction term
σ_e^2 = experimental error
r,n = rep, plot number

4. Schedule for gains from re-selection in production orchard

Re-selection was based on offspring performance measured in open-pollinated progeny tests of all orchard clones. The seed was collected in the second year of commerical harvest then tested over two years. Measurement age was assumed to be the same as age of selection in the breeding population. Rogued seed was assumed available eleven years after the first harvest for the third generation orchard. Gains from the use of family block information were added only to the extra years of the two-cycle orchard's service life.

5. Revenue from genetic gain

Genetic gains in growth were projected with a loblolly pine plantation yield model (Smith and Hafley, 1984). The assumptions were a) rotation age of 30 years (genetic gain was realized through larger trees at a set rotation length) b) 800 trees per acre, 85% initial planting survival c) genetic gains in height increased site index d) value per acre per 1% increase in site index was $47.00 for multiple end-products (pulp, chip-n-saw, sawtimber).

The value of $47.00 was obtained from multiplying the merchantable volume for each product by Timber Mart South's February 1987 market values over the range of site indexes 65 to 78 (base 25). The market value is believed to be conservative since the amount of wood per acre was projected for unthinned stands and stumpage values are expected to increase (USDA-Forest Service, 1987).

RESULTS AND DISCUSSION

Extending the service life of the second-generation orchard (two-cycle orchard) was at least as profitable as the one-cycle strategy if a) either roguing gains were at least 5.0% or b) the combined value of roguing and additional use of family-specific information was 3.0 to 4.5% (Table 2). The latter estimates were lower because roguing was an option exercised in both alternatives (for different lengths of time) whereas the additional use of family-specifc information was restricted to the second-generation orchard only.

Table 2. The minimum genetic gains from re-selection in the second generation production orchard required to favor two-cycle orchard.

	-----Gains from re-selection-----		
Roguing (%)	Additional use of family-specific data (%)	NPV one-cycle (k$)	NPV two-cycle (k$)
1	2.00	858.79	868.60
2	1.50	919.23	927.29
3	1.00	979.66	985.97
4	0.50	1040.09	1044.66
5	0.00	1100.52	1103.35

The values for gains from re-selection in the second generation production population (Table 2) constitute the minimum breeders can expect for two reasons. First, the requisite gain from re-selection will be higher than these estimates as long as the generation interval (i.e. the service life) in cooperative breeding programs continues to be longer than the biological minimum we used as our hypothetical generation interval.

Secondly, the required production population gains may be low also if there is additional cost incurred to obtain gains from additional use of family-specific information. For example, supplemental mass pollination and use of a family's site-specificity will incur costs not included in the comparision.

Sensitivity analysis
a) Gain for the third generation orchard

Genetic gain per cycle from selections in the breeding populations were considered to be 4.4% above selections in the second-generation orchard. If gain dropped to 3.7% then the two-cycle orchard strategy was optimal for generation interval of 20 years (Table 3). Genetic gain per cycle was the factor in the analysis most likely to be over-estimated in the current breeding program.

Table 3. Critical factors for the profitability of the third generation orchard after the third generation orchard and 3% gains from roguing the second generation seed orchard. Difference is between net present values of one- and two-cycle orchard strategies.

Genetic gain per cycle %	Discount rate %	Establishment costs ($)	Difference (k$)
4.4	8	101,609	111.06
3.7	8	101,609	- 6.84
4.4	9	152,413	- 1.83

The genetic gain estimate predicted for the third generation production orchard will be lower under one or more of the following conditions: a) dominance variance at the selection age is high so the phenotypic standard deviations in the gain prediction equation are underestimates (see Eq. 1) or b) the genetic value of the selections from the enrichment populations (plantation progeny) is lower than the mainline selections or c) the genetic covariances between selection and rotation ages are under-estimated by the use of age 15 genetic parameter estimates or d) if enough additional traits are included so that the selection intensity for height alone is substantially lowered.

b) Costs and annual regeneration demand

The costs reached a discount rate of 9% and an establishment cost overrun of 50% before the outcome was changed (Table 3). A reduction in the land base serviced by the third generation orchard also reduced the relative value of the one- over the two-cycle orchard (Table 4). The reduction in area serviced by the orchard slightly decreased the net profit but did not change its relative merit. This situation might be expected if vegetative propagules were used to regenerate areas previously regenerated with orchard seed. Conversely, a larger land base increases the net profit of the one-cycle orchard over the two-cycle orchard (Table 4).

Table 4. Effect of annual regeneration demand on the value of the one- and two-cycle orchard strategies for generation interval of 20 years. Difference (DIFF) is between the net present values of the one- versus two-cycle orchard strategies.

Annual regeneration (acres)	NPV one-cycle (k$)	NPV two-cycle (k$)	DIFF (k$)
16,000	2018.51	1737.13	281.38
8,000	979.66	868.60	111.06
4,000	460.23	434.30	25.93

Net profit from the traditional one-cycle orchard will continue to decline in the future if use of earlier selection continues because the orchards will have a shorter service life as well as less gain per cycle. The seed from these orchards will have less value and there will be less time to produce to seed to offset the costs of capital and establishment. The land base traditionally serviced by seed may also decrease as vegetative propagules are used to partially fulfill regeneration needs. The profitability of future one-cycle orchards will be lower than the value for third generation orchard as a result. The two-cycle orchard, by comparision, will gain appeal.

CONCLUSIONS

If the generation interval between the third and fourth generation orchard was reduced to 20 years with selection at age four years and a reduction of two years in development time then it was more profitable to bypass the third generation orchard if 1) genetic gain from selections made in the breeding population was 3.7% or lower or 2) gains from roguing the second generation orchard were 5.0% or higher or 3) gains from roguing and additional use of family-specific information were between 3.0 and 4.5%. There are other alternatives to the one-cycle orchards such as a pre-rogued orchard which may make better use of technological developments and of genetic gains from the breeding and production populations than the two-cycle orchard.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Weyerhaeuser Company and the North Carolina State University-Industry Cooperative.

LITERATURE CITED

Anonymous. 1987. Statistical Abstracts of the United States. 107th edition. U.S. Dept. of Commerce, Bureau of Census. 960 p.

Bey, C.F., D.B. Houston and R.J. Dinus. 1986. Tree genetics and improvement: III. Seed orchards. J. For. 84(5): 27-37.

Byram, T.D., W.J. Lowe, C.R. McKinley and J.F. Robinson. 1986. Thirty-fourth progress report of the cooperative forest tree improvement program. Forest Genetics Lab, Texas Forest Service, Circ. 273. 23 p.

de Steiguer, J.E. 1982. Forestland market values. J. For. 80(4): 214-216.

Duzan, H.W., C.G. Williams and E.L. O'Brien. Soil-site characteristics and the selection of genotypes for intensive management of loblolly pine. Manuscript in review.

Foster, G.S. 1986. Trends in genetic parameters with stand development and their influence on early selection for volume growth in loblolly pine. For. Sci. 32(4):1103-1254.

Gladstone, W.T. 1975. Tree improvement programs of forest industries. In Forest Tree Improv.-The Third Decade (B.Thielges, ed.). School of For. and Wildlife Mgmt., Lousiana State Univ., Baton Rouge, LA. pp. 65-74.

Greenwood, M.S. 1983. Maximizing genetic gain in loblolly pine by application of accelerated breeding methods and new concepts in orchard design. In Proc. 17th So. For. Tree Improv. Conf., Athens, GA. pp. 290-296.

Greenwood, M.S., C.C. Lambeth and J.L. Hunt. 1986. Accelerated breeding and potential impact upon breeding programs. In Proc. Workshop on Advanced Generation Breeding: Current Status and Research Needs, Baton Rouge, LA. Lousiana Agr. Exp. Stn., So. Coop. Series, Bull. No. 309. pp. 39-43.

Jett, J.B. 1983. The impact of irrigation and supplemental nitrogen fertilization on development of a young loblolly pine seed orchard. Ph. D. diss., School of Forest Resources, North Carolina State University, Raleigh, N.C. 42 p.

McKeand, S.E. and F.E. Bridgwater. 1986. When to establish advanced generation seed orchards. Silv. Gen. 35(5-6):245-247.

Smith, W.D. and W.H. Hafley. 1984. Multiproduct Yield Tables for Single-thinned Loblolly Pine Plantations. Bioeconomic Modeling Project Tech. Rep. 2, SFRC, School of For. Res., N.C.State Univ., Raleigh, N.C. 75 p.

Talbert, J.T., F.E. Bridgwater and C.C. Lambeth. 1981. Tree Improvement Cooperative Genetic Testing Manual. North Carolina State University-Industry Tree Improvement Cooperative Program, Sch. of For. Res., N.C. State University, Raleigh, N.C. 37 p.

USDA-Forest Service. 1987. The South's Fourth Forest: Alternatives for the Future. Review Draft, Government Publication Office, Washington, D.C. 365 p.

van Buijtenen, J.P. 1981. Strategies for advanced generation orchards. In Proc. 17th Can. Tree Improv. Assoc. Meet., Vancouver Is., B.C. pp. 57-64.

van Buijtenen, J.P. and W.W. Saitta. 1971. Linear programming applied to the economic analysis of forest tree improvement. J. For. 70: 164-167.

Zobel, B. and J.T. Talbert. 1983. Applied Tree Improvement. John Wiley and Sons, New York. 505 p.

POTENTIAL ALTERNATIVE INSECTICIDES FOR INSECT CONTROL IN SOUTHERN PINE SEED ORCHARDS

R. S. Cameron, G. L. DeBarr, J. F. Godbee, and J. W. Taylor[1/]

Abstract.- Azinphosmethyl, chlorpyrifos, bifenthrin, and Bacillus thuringiensis (B.t.) applied with a high-volume sprayer were evaluated for cone and seed insect control in a loblolly pine seed orchard in Texas. Aerial applications of azinphosmethyl and chlorpyrifos were compared in a loblolly pine seed orchard in South Carolina. Residues of azinphosmethyl and chlorpyrifos on pine foliage were measured in the latter study. Monthly high-volume bifenthrin and chlorpyrifos sprays provided insect control comparable to that of the azinphosmethyl standard. Compared to untreated trees, the B.t. treatment significantly reduced damage by coneworms and seedworms, but not by seedbugs. Aerial applications of azinphosmethyl reduced coneworm damage compared to the untreated check, but the chlorpyrifos sprays were ineffective. Persistence curves for aerial applications of azinphosmethyl and chlorpyrifos are presented.

Additional keywords: Coneworms, seedworms, seed bugs, Bacillus thuringiensis.

Damage surveys have shown that coneworms, Dioryctria spp., the leaffooted pine seed bug, Leptoglossus corculus (Say), and the shieldback pine seed bug, Tetyra bipunctata (Herrich-Schaffer), cause heavy losses to potential seed crops in unprotected southern pine seed orchards (Fatzinger et al. 1980). Azinphosmethyl (Guthion®), carbofuran (Furadan®), fenvalerate (Pydrin®), and permethrin (Ambush®) are specifically registered for the control of coneworms and seed bugs in southern pine seed orchards.

The availability and use of insecticides currently registered for cone and seed insect control may change dramatically in the future due to public concern about the use of pesticides and other reasons. New insecticides are needed to compensate for the disadvantages of those currently in use and to replace products which may be discontinued in the future. Three insecticides were selected for field evaluation in loblolly pine seed orchards. Chlorpyrifos was selected because it is a broad spectrum insecticide with several forestry uses already registered, it has a relatively low mammalian toxicity, and was highly toxic to D. amatella (Hulst) larvae in a laboratory bioassay (DeBarr and Fedde 1980). Bifenthrin is a second generation pyrethroid insecticide with unique chemistry, a relatively low mammalian toxicity, a potential for low application rates, a relatively long residual,

---

[1/] Respectively: Entomologist, Forest Pest Control Section, Texas Forest Service, Lufkin, TX. Research Entomologist, USDA Forest Service, SE Forest Experiment Station, Athens, GA. Pest Management Specialist, Union Camp Woodland Research, Rincon, GA. Entomologist, USDA Forest Service, Southern Region, Atlanta, GA.

and a wide spectrum of activity (including moth larvae, whiteflies, mealybugs, and mites). Bacillus thuringiensis (B.t.), a microbial insecticide considered harmless to humans and other animals including beneficial insects, has been shown to significantly reduce coneworm damage on loblolly pines in Arkansas (Gage 1975; McLeod et al. 1984).

METHODS

High Volume Ground Application Test

A hydraulic sprayer was used to spray individual trees to the point of runoff (ca. 8 gal/tree) in a loblolly pine seed orchard established in 1975 near Kirbyville, Texas. Four insecticide treatments and an unprotected control were included in this test. Azinphosmethyl (Guthion® 2S) served as a standard treatment and was applied at the recommended rate (0.2% ai). Chlorpyrifos (Dursban® 4E) was applied at the same rate as azinphosmethyl and bifenthrin (Capture® 2EC) was applied at 0.01% ai. These three conventional insecticides were applied six times at 4-week intervals from April through September in 1986. Bacillus thuringiensis (Thuricide® 32B) was applied 11 times at 2-week intervals from April through September. Since B.t. is easily washed off by rain, Bond (a sticker, extender agent) was added to the B.t. spray mix in all sprays following the second one.

Experimental design, treatment evaluation, and data analysis in this test were similar to procedures presented in detail by Nord et al. (1984). Five clones were used and treatments were randomly assigned to two ramets per clone. The distance between test trees was at least 60 ft to minimize the effects of drift. All other trees in the orchard were left untreated. Variables measured to evaluate treatment effects included April-October survival and mortality by cause among a minimum of 60 tagged conelets and cones per tree and seed quality factors (Bramlett et al. 1977) from 10 conelets and 10 cones harvested from each tree in October. Trees were inspected for the occurrence of sucking insect populations in June and October 1986 and May 1987.

Low Volume Aerial Application Test

A fixed-wing aircraft was used to apply low volume sprays to blocks of trees in a Union Camp Corporation loblolly pine seed orchard established in 1967 near Hampton, S. C. The three treatments included in this test were azinphosmethyl (Guthion® 2L), chlorpyrifos (Dursban® 4E), and an unprotected check. Azinphosmethyl was applied at the registered rate of 3 lbs ai in 10 gallons of water per acre. Chlorpyrifos was applied at the same rate and volume. Four applications were made in 1986, beginning at peak pollen release and distributed through the growing season (March 24, April 17, May 19, and July 7). Standard aerial application procedures described in detail by Nord et al. (1985) were used. Two blocks of trees were sprayed with azinphosmethyl, two with chlorpyrifos, and two were left unprotected. The treatments were randomly assigned to the six blocks. Six ramets representing six different clones in each block were selected for evaluation of efficacy. Total tree counts of sound and coneworm-damaged cones were made on each sample tree at harvest. Six sound cones per tree were dissected to determine seed quality.

Persistence of azinphosmethyl and chlorpyrifos on pine foliage was characterized in the South Carolina orchard following aerial applications in 1986 and 1987. Dursban® 4E was applied in 1986, and Dursban® 50W was used in 1987. Guthion® 2L was applied both years. Composite samples of needles from one branch on five trees in each spray block were collected at 0, 2, 4, 8, 16, and 32 days post-application and subjected to residue analysis procedures described by Bush et al. (1986). Persistence curves were developed from combined data for the four applications in 1986 and initial deposits were determined for the first application made on April 2, 1987.

Data Analysis

Data were analyzed by the General Linear Model and Duncan's New Multiple Range Test procedures using the Statistical Analysis System (Ray 1982). Treatments were assumed to be fixed while clones and ramets within clones were assumed to be random effects in a mixed linear model. Only those variables which had a significant F value ($P < 0.05$) for treatments were subjected to Duncan's New Multiple Range Test. Percentage data were transformed using the arc sine square root transformation.

RESULTS AND DISCUSSION

Ground Application Test - Texas

Significant differences ($P < 0.01$) among treatment means were observed for all factors, except percent other cone damage and percent second-year aborted ovules (Tables 1 and 2). Azinphosmethyl, bifenthrin, and chlorpyrifos all provided excellent cone and seed insect control. No significant differences were detected between the azinphosmethyl standard and bifenthrin treatments for any of the factors measured. Near 100% survival of conelets, cones, and ovules in conelets was attained with the bifenthrin treatment. Thus, nearly all the mortality observed on the other treatment trees can be attributed to insect damage. Chlorpyrifos was less effective than azinphosmethyl and bifenthrin for controlling seed bugs. Significant differences between chlorpyrifos and the latter two treatments occurred for mean percent sound ovules in conelets and percent total insect damage in cones (Table 2). These observations are supported by laboratory results which indicated that chlorpyrifos is less toxic than other insecticides to the leaffooted pine seed bug (DeBarr and Nord 1978; Nord and DeBarr 1983).

Compared to the unprotected check, B.t. significantly reduced coneworm damage among conelets and cones and seedworm, Cydia toreuta (Grote), damage in cones (Tables 1 and 2). B.t. control of coneworm damage among conelets (mostly D. clarioralis (Walker) damage) was as good as that provided by the conventional insecticides. However, the conventional insecticides were more effective than B.t. for controlling coneworm damage, caused mostly by D. amatella, to second year cones. Also, B.t. did not reduce conelet mortality, ovule abortion, or seed bug-damaged seeds as compared to untreated checks, reflecting a total lack of seed bug control. This was not unexpected since Thuricide® 32B is known to be effective only against lepidopterous larvae.

Table 1. Mean percent survival, coneworm damage, and total mortality among conelets and cones on trees treated with one of four high volume sprays or left unprotected in a loblolly pine seed orchard near Kirbyville, TX, during 1986.

Treatment[1/]	Conelets			Cones		
	Apr-Oct survival	Coneworm damage	Other mortality	Apr-Oct survival	Coneworm damage	Other mortality
Azinphosmethyl	98.1a[2/]	0a	1.9a	95.8ab	2.0a	2.2
Bifenthrin	99.5a	0a	0.5a	99.0a	0.5a	0.5
Chlorpyrifos	96.0a	0.1a	3.9ab	94.5ab	0.9a	4.6
B. thuringiensis	86.1b	0.2a	13.7c	92.0bc	6.2b	1.8
Unprotected	82.8b	4.9b	12.3bc	82.0c	17.5c	0.5

[1/] Formulations (rates): Guthion® 2S (0.2% ai), Capture® 2EC (0.01% ai), Dursban® 4E (0.2% ai), and Thuricide® 32B (16 BIU/100 gal).

[2/] Means within each column followed by no letter or the same letter are not significantly different ($P > 0.05$, ANOVA, Duncan's Multiple Range Test).

Table 2. Mean percentages for seed quality factors on trees treated with one of four high volume sprays or left unprotected in a loblolly pine seed orchard near Kirbyville, TX, during 1986.

Treatments[1/]	Conelets	Cones				
	Sound ovules	Filled seed	2nd-yr aborted ovules	Seedbug damaged	Seedworm damaged	Total insect damage
Azinphosmethyl	99.3a[2/]	81.6ab	0.4	0.6a	0a	1.0ab
Bifenthrin	99.4a	83.0a	0.1	0.4a	0a	0.5a
Chlorpyrifos	95.3b	83.4a	0.4	0.9a	0a	1.3b
B. thuringiensis	72.2c	74.4c	2.0	3.7b	0.1a	5.8c
Unprotected	79.8c	77.6bc	0.5	3.0b	0.7b	4.3c

[1/] Formulations (rates): Guthion® 2S (0.2% ai), Capture® 2EC (0.01% ai), Dursban® 4E (0.2% ai), and Thuricide® 32B (16 BIU/100 gal).

[2/] Means within each column followed by no letter or the same letter are not significantly different ($P > 0.05$, ANOVA, Duncan's Multiple Range Test).

The eleven bi-weekly full coverage B.t. sprays applied at a relatively high rate did not provide excellent control of D. amatella in this study. However, B.t. may be more effective and practical for the control of a single generation pest requiring a narrow window of protection, such as D. disclusa (Heinrich).

Very few mealybugs and scale insects were observed on any of the test trees during inspections conducted in June and October 1986 and May 1987 in the Texas seed orchard. No sucking insect population outbreaks were associated with any of the treatments in this test.

Aerial Application Test - South Carolina

Azinphosmethyl aerial sprays significantly reduced damage by all Dioryctria species combined, but chlorpyrifos failed to reduce coneworm attacks (Table 3). Neither chlorpyrifos nor azinphosmethyl prevented attacks by D. amatella or D. merkeli Mutuura and Munroe early in the summer. Azinphosmethyl significantly reduced late attacks by D. amatella, while chlorpyrifos did not. No differences in seed yields nor seed quality factors were detected in the aerial spray study. Insect-caused damage was low and yields were high for all treatments, including the unprotected control (Table 4). Thus, there was little opportunity for the insecticide treatments to improve seed yields and seed quality.

Residues of chlorpyrifos detected immediately following the 1986 applications were very low (Fig. 1). These initial deposits were below the level determined to be effective for the leaffooted pine seed bug in bioassays for azinphosmethyl (10 ppm, DeBarr and Nord, unpubl. data). Azinphosmethyl residues were more acceptable, but initial deposits were only about one-half the deposits of 175-200 ppm detected on foliage treated with low volume or aerial applications in previous studies (DeBarr and others, unpubl. data).

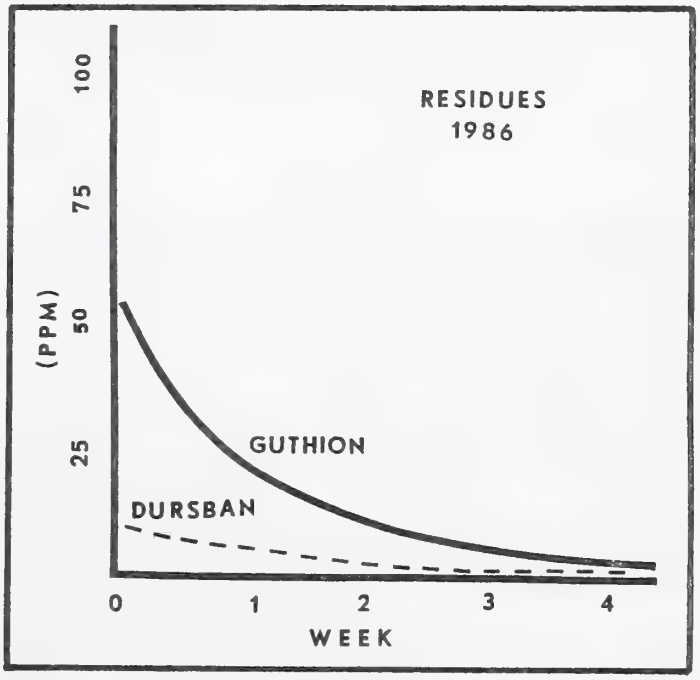


Figure 1. Persistence curves for foliar residues of chlorpyrifos (Dursban® 4E) and azinphosmethyl (Guthion® 2L) following fixed-wing aircraft applications to a loblolly pine seed orchard in South Carolina.

Apparently, most of the chlorpyrifos formulated as an emulsifiable concentrate volatilized before it was deposited on the foliage. Assuming an LC90 of 10 ppm is required for effective cone and seed insect control, sufficient amounts of azinphosmethyl were present on the pine foliage for about three weeks post-treatment in 1986. The initial residues of chlorpyrifos following the aerial application of the wettable powder formulation (Dursban® 50W) in April of 1987 averaged 30 ppm. This is about four times those resulting from the use of the emulsifiable concentrate (Dursban® 4E) the previous year. However, the average initial deposit of azinphosmethyl was about 60 ppm, which was similar to residues found in initial foliar deposits the previous year.

Table 3. Mean percentages of cones infested by Dioryctria on loblolly pines receiving aerial azinphosmethyl or chlorpyrifos sprays or left unprotected in a seed orchard in Hampton County, S.C. during 1986.

Treatment[1/]	Dioryctria damage-total	D. merkeli damage	D. amatella green cones	D. amatella dead cones
Azinphosmethyl	12.4a[2/]	8.2	2.7	1.5a
Chlorpyrifos	16.5b	8.5	4.9	3.0b
Unprotected	15.0b	9.5	3.1	2.3b

1/ Formulations (rates): Guthion® 2L (3 lb ai/ac), Dursban® 4E (3 lb ai/ac).

2/ Means within each column followed by no letter or the same letter are not significantly different (P > 0.05, ANOVA, Duncan's Multiple Range Test).

Table 4. Mean seed yields per cone and seed quality for loblolly pines receiving aerial azinphosmethyl or chlorpyrifos sprays or left unprotected in a seed orchard in Hampton County, SC, during 1986.

Treatment[1/]	Number filled seed/cone	Percent filled	Percent empty	2nd-yr aborted ovules	Total seedbug-damaged seed
Azinphosmethyl	84.4	72.5	24.7	0.1	1.1
Chlorpyrifos	81.5	73.5	21.5	0.5	2.2
Unprotected	74.3	70.4	25.5	0.5	1.5

1/ Formulations (rates): Guthion® 2L (3 lb ai/ac), Dursban® 4E (3 lb ai/ac).

2/ Means within columns are not significantly different (P > 0.05, ANOVA).

CONCLUSIONS

High volume ground sprays of bifenthrin, chlorpyrifos, and azinphosmethyl provided excellent control of coneworms and seed bugs. Bifenthrin applied at a low concentration, 0.01% ai, was consistently the best treatment. Chlorpyrifos was less effective than azinphosmethyl and bifenthrin for controlling seed bugs. Bacillus thuringiensis significantly reduced coneworm and seedworm damage, but was less effective than the three conventional insecticides for coneworm control and did not reduce seed bug damage. Sucking insect outbreaks were not associated with any of the treatments in this study.

Insect control in the 1986 aerial spray test was disappointing. Seed bug damage was light in all treatments, allowing for no significant treatment differences. Coneworm damage was reduced by the azinphosmethyl treatment, but chlorpyrifos was ineffective. Residues of chlorpyrifos applied as an emulsifiable concentrate in 1986 were below those needed to control cone and seed insects. Residues of chlorpyrifos applied as a wettable powder in 1987 were about four times those detected in 1986, but were still only one-half the quantity detected for azinphosmethyl.

Results reported here strongly support further testing of bifenthrin and chlorpyrifos in pine seed orchards. The wettable powder formulations of both these insecticides should be utilized in future tests. Bifenthrin needs to be tested at a lower rate and as a low volume spray. The wettable powder formulation of bifenthrin (Talstar®10WP) should be tested in future studies since this is the product being developed by FMC Corporation for ornamental trees and shrubs.

Old insecticide registrations are in constant jeopardy. Fewer and fewer new insecticides are becoming available each year because of increased development costs. New registrations are increasingly difficult to obtain, particularly for minor uses such as for seed orchards. Continued support from seed orchard managers and tree improvement cooperatives is essential if alternative insecticides for cone and seed insect control are to be registered.

ACKNOWLEDGMENTS

The insecticides used in this study were provided as follows: Guthion® by Mobay Chemical Corporation, Dursban® by Dow Chemical Company, Thuricide® by Sandoz Crop Protection Corporation, and Capture® by FMC Corporation. Funds for this study were provided by the Texas Forest Service, USFS - Region 8, Dow Chemical Company, and Sandoz Crop Protection Corporation. Thanks are extended to B. Upton and C. Ware, Texas Forest Service; J. Seckinger and J. Sullivan, Union Camp Corp.; and A. Hylton and M. Cody, USFS for their assistance with data collection. P. Bush, University of Georgia, provided the residue analyses. W. Lowe and C. McKinley, Texas A&M Univ., and L. Rayburn, Stephen F. Austin State Univ., assisted with statistical analyses.

LITERATURE CITED

Bramlett, D. L., E. W. Belcher, Jr., G. L. DeBarr, G. D. Hertel, R. P. Karrfalt, C. W. Lantz, T. Miller, K. D. Ware, and H. O. Yates, III. 1977. Cone analysis of southern pines - a guide book. USDA For. Ser. Gen. Tech. Rep. SE-13. 28 pp.

Bush, P. B., D. G. Neary, J. W. Taylor, Jr., and W. L. Nutter. 1986. Effects of insecticide use in a pine seed orchard on pesticide levels in fish. Water Resources Bull. 22: 1-11.

DeBarr, G. L. and V. H. Fedde. 1980. Contact toxicity of 17 insecticides to larvae of Dioryctria amatella (Lepidoptera: Pyralidae). Can. Entomol. 112: 521-523.

DeBarr, G. L. and J. C. Nord. 1978. Contact toxicity of 34 insecticides to second-stage nymphs of Leptoglossus corculus (Hemiptera: Coreidae). Can. Entomol. 110: 901-906.

Fatzinger, C. W., G. D. Hertel, E. P. Merkel, W. D. Pepper, and R. S. Cameron. 1980. Identification and sequential occurrence of mortality factors affecting seed yields of southern pine seed orchards. USDA For. Ser. Res. Pap. SE-216. 43 pp.

Gage, E. V. 1975. Evaluation and timing of selected insecticides for control of Dioryctria coneworms in a loblolly pine seed orchard. M. S. thesis, Univ. of Arkansas, Fayetteville. 28 pp.

McLeod, P. J., W. C. Yearian, and S. Y. Young. 1984. Evaluation of Bacillus thuringiensis for coneworm, Dioryctria spp., control in southern pine seed orchards. J. Georgia Entomol. Soc. 19: 408-413.

Nord, J. C. and G. L. DeBarr. 1983. Contact toxicities of 12 insecticides to Leptoglossus corculus adults. Can. Entomol. 115: 211-214.

Nord, J. C., G. L. DeBarr, N. A. Overgaard, W. W. Neel, R. S. Cameron, and J. F. Godbee. 1984. High-volume applications of azinphosmethyl, fenvalerate, permethrin and phosmet for control of coneworms (Lepidoptera: Pyralidae) and seed bugs (Hemiptera: Coreidae and Pentatomidae) in southern pine seed orchards. J. Econ. Entomol. 77: 1589-1595.

Nord, J. C., G. L. DeBarr, L. R. Barber, J. C. Weatherby, and N. A. Overgaard. 1985. Low-volume applications of azinphosmethyl, fenvalerate, and permethrin for control of coneworms (Lepidoptera: Pyralidae) and seedbugs (Hemiptera: Coreidae and Pentatomidae) in southern pine seed orchards. J. Econ. Entomol. 78: 445-450.

Ray, A. J. (ed). 1982. SAS Users' Guide: Statistics. SAS Institute, Cary, North Carolina. 584 pp.

SEED YIELDS PER BUSHEL: ORCHARD VS NATURAL STANDS

K. O. Summerville [1/]

Abstract.--Seed yields per bushel for loblolly (Coastal Plain and Piedmont North Carolina Provenance), longleaf, Eastern white pine, and Fraser fir are documented. North Carolina Division of Forest Resources orchard yields are compared with yields of commercial collections from natural stands in North Carolina. Orchard seed yields per bushel are greater than natural stands for loblolly, longleaf and Fraser fir.

Additional Keywords: Pinus palustris Mill., Pinus strobus L., Pinus taeda L., Abies fraseri (Pursh) Poir.

Cones from stands within the species natural geographic zones in North Carolina were used for this comparison. The natural range of loblolly (Pinus taeda L.), longleaf (Pinus palustris Mill.), Eastern white pine (Pinus strobus L. and Fraser fir (Abies fraseri (Pursh) Poir.) are illustrated on the map of Figure 1 (Little 1971). Seed orchards of these four species are located within the natural range of each species except for Fraser fir. The Fraser fir orchard is at a lower elevation (3400 feet) than naturally occurring stands. Fraser fir begins to occur mixed with red spruce at 4500 feet elevation on some sites in the Great Smoky Mountains National Park and at 5000 feet on Roan Mountain. As elevations increase, Fraser fir becomes the dominant component of the tree canopy. (Holmes 1911, Whittaker 1956, Mark 1958, Crandall 1958)

Early discussions of the value of orchard production over natural stand production have given greater consideration to potential genetic value over that of increased seed yield per bushel of purchased or harvested cones. (Perry and Wang 1958, Zobel et al. 1958). At the early stages of orchard development, data was not available to compare orchard tree yields with natural stands because of the young age of orchard trees. This presentation will document the yields of 20 year old orchard trees with those of natural stands for the above four species.

METHODS

The natural stand seed collection data is taken from cone purchase and clean seed inventories of 1978-81 (Table 1). The orchard seed collection data is taken from cone harvest and clean seed extraction inventories of 1981-84 (Table 2). Although the years of harvest between natural stand and orchard are different, this does not prevent comparing the data. From the seed yields expressed in Table 3 (Schopmeyer 1974), it is evident that yields of North

---

[1/] Staff Forester Tree Improvement, North Carolina Division of Forest Resources, Clayton, North Carolina.

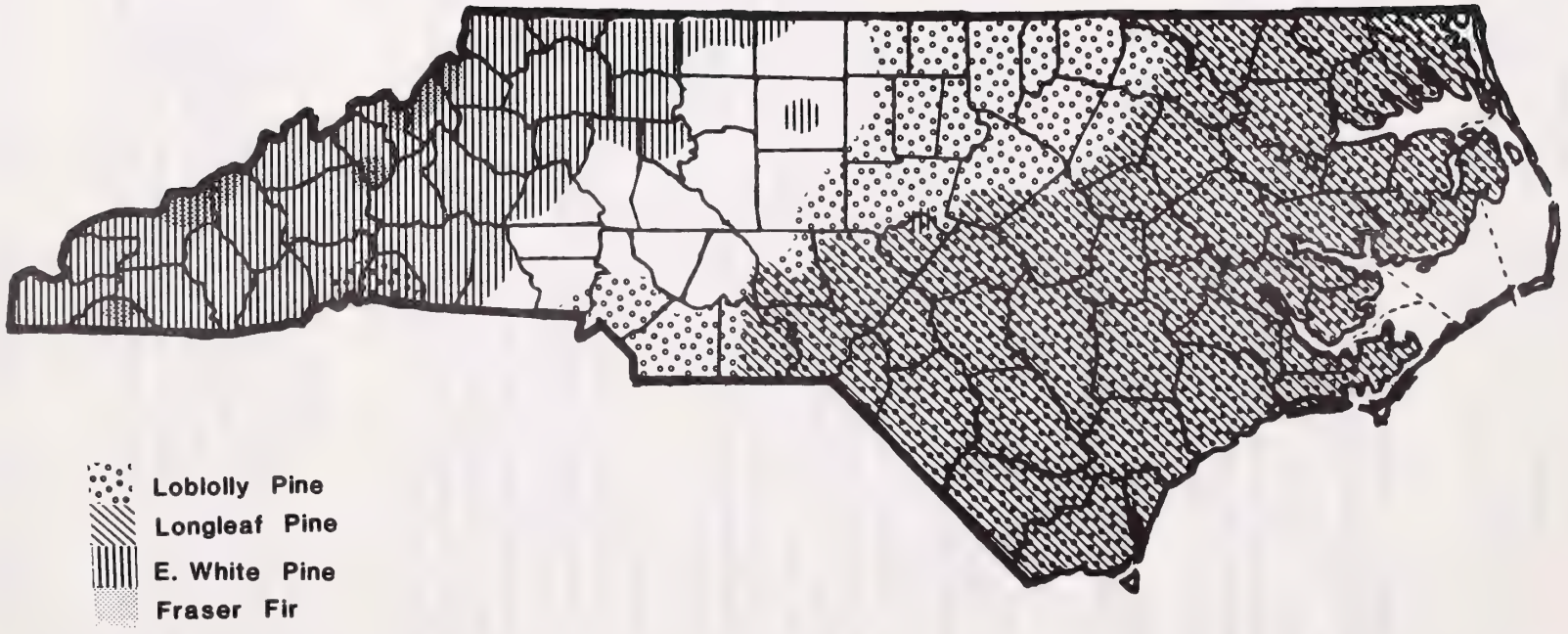


Fig. 1 Species Natural Range in North Carolina

Carolina natural stand sources (Table 1) are within previously reported limits of the total range for these four species. Results of nine years (1978-86) of cone/seed yields (1.47 pounds of seed per bushel average) for loblolly pine reported by the North Carolina State Industry-Cooperative Tree Improvement Program indicate that the North Carolina orchard yield data presented in Table 2 is comparable. (North Carolina State University 1987)

Table 1.--Bushels of cones and pounds of seed yields of natural stands in North Carolina[2]

	Loblolly Pine				Longleaf Pine		Eastern White Pine		Fraser Fir	
	Piedmont		Coastal Plain							
Year	Bu	Lb	Bu	Lb	Bu	Lb	Bu	Lb	Bu	Lb
1978	390	126	703	444	113	43	3054	1761	332	694
1979	764	432	637	1010	2575	2141	141	59	NO CROP	
1980	291	403	3420	1492	2515	1829	52	123	NO CROP	
1981	51	27	1158	1687	7732	6182	5111	3490	2442	4710
TOTAL	1496	988	5918	4633	12935	10195	8358	5433	2774	5404
AVG	374	247	1480	1158	3234	2549	2090	1358	694	1351
LB/BU	.66		.78		.79		.65		1.95	

Table 2.--Bushels of cones and pounds of seed yields of seed orchard crops in North Carolina[2]

	Loblolly Pine				Longleaf Pine		Eastern White Pine		Fraser Fir	
	Piedmont		Coastal Plain							
Year	Bu	Lb	Bu	Lb	Bu	Lb	Bu	Lb	Bu	Lb
1981	1284	2244	885	1630	1193	1617	658	298	148	336
1982	442	862	233	337	726	726	NO CROP		54	166
1983	228	302	283	373	570	558	957	455	101	286
1984	898	1145	1081	1365	814	769	5004	3633	218	702
TOTAL	2852	4553	2482	3705	3303	3670	6619	4386	521	1490
AVG	713	1138	621	926	826	918	1655	1097	130	373
LB/BU	1.60		1.49		1.11		.66		2.87	

[2] Cone/seed data taken from cone purchase and orchard harvest records of North Carolina Division of Forest Resources, Nursery/Tree Improvement Program

Table 3.--Pound of seed per bushel as reported in Seeds of Woody Plants in the United States

Loblolly Pine	Longleaf Pine	Eastern White Pine	Fraser Fir
.6 - 1.3	.8	.3 - 1.7	2 - 3

RESULTS

Orchard seed yields per bushel of cones for Piedmont loblolly pine is .94 pounds greater than natural stand production and Coastal Plain loblolly is .71 pounds greater. Longleaf orchard yield is .32 pounds higher than natural stand production. White pine orchard yield (pounds of seed per bushel) is only .01 more than natural stand production. Fraser fir orchard production is .92 pounds greater than the natural stand production.

DISCUSSION AND CONCLUSION

With the exception of Eastern White pine these orchard seed yields are an improvement over natural stands. There may be several reasons for the low orchard seed yield for Eastern white pine in the North Carolina orchard. Insects (white pine cone beetle, Conephthorus coniperda, Schwarz and cone borer, Eucosma tocullionana, Heinrich) have caused a reduction or complete loss of cone crops. Currently available insecticides do not provide adequate protection from these two insects. Cones are harvested without regard to clonal variation in cone ripeness. Eastern white pine cones that are not ripe when harvested will case harden and not open during seed extraction processes. Harvesting by clonal groups that ripen near the same time should improve seed yield.

Longleaf seed yields have been increased by a foliar spray mixture of cytokinin and boric acid (Hare 1987). This spray also is reported to reduce the amount of conelet abortion that happens with longleaf pine. Including this spray application in the cultural practices of longleaf orchard operations could improve yields by one half.

The loblolly seed yields are not up to the two pound per bushel reported for some orchards in the 31st Annual Report of the North Carolina State University Cooperative. More complete monitoring for seed bug damage may indicate a reason for some of the lower yield per bushel.

At this time the Fraser fir seed orchard at Crossnore, North Carolina is the only one that is old enough to produce cones and seed for which records are available. In the future younger clonal orchards should provide data for comparison. Insects other than seed chalcids have not been identified as causing cone or seed loss in Fraser fir. (Hedlin, et al. 1980)

LITERATURE CITED

Brown, D. M. 1941. Vegetation of Roan Mountain: A Phylosociological and Successional Study. Ecol. Monogr. 11:1, pp 61-97.

Crandall, D. L. 1958. Ground Vegetation Patterns of the Spruce-Fir Area of the Great Smoky Mountains National Park. Ecol. Monogr. 28:4 pp 337-360

Hare, R. C. 1987. Increase Longleaf Pine Seed Yields by Inhibiting Conelet Abortion. South. Jour. of Appl. For. 11:1 pp 6-9.

Hedlin, A. F., H. O. Yates, III, D. C. Tovar, B. H. Ebel, T. W Koerber and E.P. Merkel. 1980. Cone and Seed Insects of North American Confiers. Can. For. Ser. U.S.F.S., Sec. deAgric. yRec. Hidraulicos, Mex. p 122.

Holmes, J. S. 1911. Forest Conditions in Western North Carolina. N. C. Geo. and Econ. Survey, Bulletin #23.

Little, E. L., Jr. 1971. Atlas of United States Trees: Vol. 1, Conifers and Important Hardwoods. USDA:FS, Misc. Pub. No. 1146, Washington, D. C.

_____ 1979. Checklist of United States Trees (native and naturalized). USDA, Agric. Handbook 541, 375 p.

Mark, A. F. 1958. The Ecology of the Southern Appalachian Grass Balds. Ecol. Monogr. 28:4 pp 293-336.

North Carolina State University. 1987. Thirty-first annual report, N. C. State University-Industry Cooperative Tree Improvement Program. Sch. Forest Resources, Raleigh, N. C. 55 p.

Perry, T. O. and C. W. Wang. 1958. The Value of Genetically Superior Seed. Jour. of For. 56:11, pp 843-845.

Schopmeyer, C. S. 1974. Seeds of Woody Plants in the United States. USDA:FS, Agric. Handbook 450, 883 p.

Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. Ecol. Monogr. 26:1 pp 1-80.

Zobel, B. J., J. Barber, C. L. Brown and T. O. Perry. 1958. Seed Orchards - Their Concept and Management. Jour. of For. 56:11, pp 815-825.

THE WEISS-MCNAIR CONE HARVESTER

G.A. Lowerts and M.H. Zoerb, Jr.[1/]

Abstract. -- The Weiss-McNair cone harvester is a gasoline powered machine that scoops up slash (Pinus elliottii Englem. var. elliottii) and loblolly (P. taeda L.) pine cones from the orchard floor using two paddle wheels. The cones are then moved via two conveyor belts to a 20 bushel crate on a trailer at the rear of the machine. An air blower removes pinestraw and small sticks that were picked up with the pine cones.

INTRODUCTION

The harvest of pine cones is the most expensive aspect of seed orchard management. Maximum benefit from orchard management practices can only be achieved if the cone harvest is conducted in a timely and efficient manner. Because of the high cost of harvesting pine cones and the increasing potential for large cone crops due to enhanced management practices and increasing orchard tree size, the Company has examined and tested several cone harvesting systems. This paper describes one system which appears to have potential for increasing harvest efficiency.

PINE CONE HARVESTING PROCEDURE

Slash pine cones are dislodged with a tree shaker. Loblolly pine cones are hand picked by two-man crews in aerial lift trucks. Regardless of the collection method, the pine cones are removed from each tree and dropped to the ground. Moving the pine cones from the orchard floor to the seed extraction facility has been the most time consuming, and tedious aspect of cone harvest.

Prior to 1984, pine cones were removed from the orchard floor by hand and deposited into 20 bushel capacity cone crates by temporary labor crews. The temporary work force varied with the size of the cone crop, but was consistently increasing year by year. In order to reduce the cost and shorten the cone harvest season, a mechanical method of retrieving pine cones from the orchard floor and transferring to 20 bushel crates was developed.

THE WEISS-MCNAIR CONE HARVESTER

The Weiss-McNair cone harvester was developed from a pecan harvester design. After several prototypes and many modifications, the cone harvester has become an efficient and reliable machine (For specifications, see Appendix A). All producing seed orchards are mowed a few days before cone harvest to

---

[1/] Seed Orchard Manager and Tree Improvement Project Leader, respectively, Woodlands Research Department, Union Camp Corporation, Rincon, Georgia 31326 USA.

facilitate the operation of the cone harvester. During the harvest, the machine operator begins cone pickup next to an orchard tree. The tight turning circle of the machine allows the operator to move in a tight spiral around the tree (Figure 1). On the average, three to five slash pine cones remain on the ground after the machine moves around a tree. Loblolly cones are generally smaller than slash cones and seven to ten cones are left on the ground. One person then follows the machine and picks up by hand any remaining cones.

Pine cones on the orchard floor are picked up by two paddle wheels in the machine pickup head. The cones are then transferred via two conveyor belts to a 20 bushel crate on a small trailer towed behind the machine. Light weight trash such as mowed grass, pine needles, and small sticks are removed from the pine cones when the cones pass through a high speed air stream prior to their deposition in the crate. Large sticks and other heavy objects are not removed. The machine is powered by a 30 horsepower air-cooled engine and all mechanical systems are hydraulically driven including the pickup head which automatically adjusts (floats) to small contour changes in the orchard floor. The pickup head will not collect pine cones in holes or other depressions in the orchard floor. The machine has two front drive wheels and a single steering wheel in the rear. Increased efficiency and speed of cone pickup off the orchard floor are among the advantages of using the mechanical cone harvester. The greatest benefit is the reduction in labor costs since fewer temporary employees are needed.

Detailed information on the cone harvester mechanical specifications and current cost of the machine are available from:

Fred Parks
Albany Tractor Co.
1709 South Slappy Blvd.
Albany, Georgia 31701
(912) 432-7468

The Weiss-McNair cone harvester is manufactured by:

Weiss-McNair Incorporated
531 Country Drive
Chico, CA 95926
(916) 891-6214

Figure 1. A typical cone harvest pattern around an orchard tree.

Appendix A. Weiss-McNair Cone Harvester Specifications.

GENERAL

Engine - - Wisconsin Model VH4D; 30 h.p. @ 2800 RPM

Transmission - - Eaton Hydrostatic

Hydraulic Pump - - Sperry Rand

Power Drive Wheels - - Two 26 X 12.00 X 12 Knob Grip Tires

Steering Rear Wheel - - One 18 X 9.50 X 8 Turf-Saver

Hydraulic Oil - - 12 gallon capacity

Gasoline - - 12 gallon capacity

Fuel Consumption - - 2 gallons per hour

FLOATING PICKUP HEAD

Size - - Five Feet Wide; Three Feet High; Three Feet Long

Paddle Wheels - - One 30 in. Diameter with 5 in. Rubber Paddles
- - One 10 in. Diameter with 5 in. Rubber Paddles

Conveyer Belt - - One 5 ft. X 12 in.; rubber

Tires - - Two 13 X 6.50 X 6

CONVEYER TO CONE CRATE

Conveyer Belt - - one 12 ft. X 10 in.; chain link

Blower Fan - - 24 in. X 9 in. Paddle Fan

Air Duct - - 12 in. Diameter To 5 in. X 10 in. Air Shoot

Metal Cone Slide - - 16 in. X 30 in. Metal Slide @ 45^{o} Angle To Crate

CONE CRATE TRAILER

Capacity - - One 20 Bushel Crate

Trailer - - Metal Frame

Tires - - Two 4.80 X 8

SEED ORCHARD MANAGEMENT II

MODERATED BY DR. BRUCE BONGARTEN

University of Georgia

PHENOTYPIC SELECTION EFFECTIVE IN A NORTHERN RED OAK SEEDLING SEED ORCHARD

T. La Farge and R. A. Lewis [1/]

Abstract.--In 1984 a progeny test of 220 northern red oak open-pollinated families, established in 1973 by the Tennessee Valley Authority, was converted to a seedling seed orchard. Located on the Watauga District of the Cherokee National Forest, this orchard was measured for seven traits in August, 1985 at age 13 years. Significant differences ($p \leq 0.001$) were found among families for height, DBH, straightness, forking, D^2H, survival, and defoliation. Single tree heritabilities ranged from 0.113 for D^2H to 0.375 for percent survival. Family heritabilities ranged from 0.240 for D^2H to 0.711 for survival. On the basis of these results, 21 families have been rogued, and all families have been thinned to the best one or two trees in each of eight four-tree-row plots. Selection differentials and genetic gains showed that positive gains obtained from single tree selection were greater than those obtained for family selection. It is concluded that phenotypic selection in plantations will work well in northern red oak.

Additional keywords: Quercus rubra L., seedling seed orchard, heritability, selection differential, genetic gain, roguing.

Northern red oak (Quercus rubra L.) is among the most important of the northern hardwoods, and it attains the largest size of the eastern upland oaks (Core 1971). Its range extends from New Brunswick, southern Ontario, and Minnesota south to Alabama and Georgia. This species is most frequently found in coves and on lower and middle slopes having northern and eastern aspects. It grows best on fine textured soils and topographies favoring a high water table.

Planting red oak and establishing a stand is not difficult if done on a suitable site. Some plantings in the southern Appalachians have averaged better than 90 percent survival (Russell 1971). Early provenance tests have indicated that differences among geographic sources are less than differences among trees within sources, so that single tree selection would seem to have the greatest potential for genetic gain (Cech 1971).

The present study was undertaken after a decision was made to convert a progeny test to a seedling seed orchard. The trees were measured, seven traits were analyzed, and the test was rogued and thinned as the first stage of selection. The procedures used and genetic gains attained are reported herein.

1/ Eastern Zone Geneticist and Supervisory Geneticist, respectively, U.S.D.A. Forest Service, Region 8, Atlanta, Georgia. The authors wish to express their appreciation to Ken Proffit, Seed Orchard Manager of the Northern Red Oak Seed Orchard, Tom Crews, Watauga District Silviculturist, and B. D. Barr, Watauga District Ranger. Without considerable help in measuring, thinning, and caring for this orchard by these people, this study would not have been possible.

HISTORY

The Tennessee Valley Authority (TVA) began a breeding program for the oaks in the early 1960s and initially used mass selection of superior phenotypes. However, by the late 1960s data obtained from hardwood progeny tests in the South had demonstrated that phenotypic mass selection is not effective for growth traits. Hence, they decided to establish a large breeding population of open-pollinated families. This population was to provide data on geographic variation and plant material to be used to produce seed orchards. To accomplish this, the tree improvement staff at TVA selected a representative sample of dominant and codominant trees having good form from several geographic sources (Farmer 1980).

In the spring of 1973 the TVA planted a progeny test of 220 open-pollinated northern red oak families on the Watauga Ranger District of the Cherokee National Forest in Tennessee. The field design was eight replications of four trees of each progeny in row plots at a 10 X 10-foot spacing. This test was one of 11 plantations. The other 10 plantations are located in Kentucky, Tennessee (3), Alabama, Georgia, North Carolina, Illinois, Indiana, and Ohio. The seed sources used represented all of these states except Georgia and Ohio.

The progeny test area is located in the Dry Hill section of the Watauga Ranger District and is a quarter of a mile from Watauga Lake. The ecological management unit is on broad ridgetops and upper slopes of low lying intermountain ridges having elevations of less than 2,500 feet. The soil texture is a medium silty clay loam to clay loam that grades to clay at depths of three to five feet. Depth to the underlying limestone bedrock ranges between six and 12 feet. The average soil moisture regime is dry. Slopes vary from two to 35 percent, but most are between six and 20 percent. The test area occupies 16.2 acres.

The TVA performed annual maintenance of the test by disking or mowing until 1979, when they dropped funding for the project. In 1983 the Watauga District resumed annual mowing to prevent weed-tree species from taking over the site. Deer browsing, rodents, and mowing accidents have taken their toll, and growth has been slowed by a heavy sod of fescue grass. Overall survival was 44.4 percent as of September, 1985, when the measurements for the current data analyses were taken.

In 1982 the TVA asked Region 8 to assume full responsibility for the test. Region 8 accepted and decided to thin and rogue the test to convert it to a seedling seed orchard. Although height and diameter measurements were made in 1982 at age 10, a more complete set of measurements was made at age 13.

METHODS

Six traits were measured in August and September of 1985. They were: (1) height to the nearest foot; (2) diameter at breast height (DBH) to the nearest inch; (3) straightness, by an index of 1 = crooked to 6 = straight; (4) forking, by an index in which 1 = 3 or more forks, 2 = 2 forks, 3 = 1 fork, and 4 = 0 forks; (5) insect defoliation, by an index of 0 = no defoliation, 1 = partial defoliation, and 2 = complete defoliation; and (6) apical dominance, by an index in which 1 = no dominance and 2 = complete dominance. Apical

dominance was later dropped from consideration after it proved impossible to estimate subjectively. The indexes used in this study are similar to many used in biology, despite questions about their frequency distributions (Sokal and Rohlf 1969).

Two additional traits were included in the analyses: percent survival and D^2H, the latter trait being computed from the DBH and height measurements as an approximation of single-tree volumes. Percent survival was also analyzed as arcsine X (square root of proportion), but this transformation accomplished no reduction in mean square error of the analysis of variance and was, therefore, dropped.

All data were analyzed by a one-way analysis of variance on a UNIVAC 1100/84 computer with the BioMedical Data Processing (BMDP) package, program P7D (Dixon 1983). Selection differentials, phenotypic and genotypic standard deviations, selection intensities, and genetic gains were all calculated by methods given by Becker (1984). All analyses had to be performed in two overlapping data sets, since BMDP program P7D could not handle the size of the complete data set. Therefore, blocks 1 - 6 were analyzed as Data Set #1, and Blocks 1,2,3,4,7,8 were analyzed as Data Set #2, blocks 1 - 4 being common to both sets. Results from both data sets are listed in all tables.

After analysis, we decided to use all seven traits to identify the poorest families, so that the orchard could be rogued and thinned with a combined family and single tree selection. The roguing system used was a variant of the independent culling levels selection method and was accomplished by means of a spread sheet. Threshold limits were arbitrarily chosen for each trait so that they fell toward the lower limit of the range of variation of all families. Any family which fell below these limits for at least three traits was marked for roguing. On this basis 21 of the 220 families were rogued from the orchard.

Single tree selection was accomplished by thinning all but one, or occasionally two, trees from each plot. In a very few cases no trees were left in a plot, because none appeared capable of survival. Of course, many plots were already empty, since survival was only 44.4 percent.

RESULTS

The means and numbers of observations of the seven traits analyzed are listed in Table 1. At age 13 tree height ranged from one to 26 feet and DBH from zero to six inches. All except one of the seven traits showed significant differences among families at the 0.001 probability level in both data sets (Table 2). The family heritabilities are somewhat low, but the single tree heritabilities, while not high, are in the usual range reported for most species (Table 3). The heritabilities for height and survival are especially encouraging.

Once all roguing and thinning had been accomplished and all remaining trees identified, it was possible to calculate selection differentials and intensities of selection. These are listed for families in Table 4 and for single trees in Table 5. The percentage genetic gains for family, single tree, and combined selection (Table 6) were obtained from the appropriate heritabilities and selection differentials.

Table 1.--Means and numbers of observations for seven traits in two data sets of a northern red oak seedling seed orchard

Trait	Data Set: # 1 Mean	# 1 Number of trees	# 2 Mean	# 2 Number of trees
Height, feet	11.64	2,379	11.17	2,309
DBH, inches	1.52	2,379	1.46	2,309
Straightness, index	2.20	2,380	2.15	2,310
Forking, index	1.71	2,379	1.67	2,309
D^2H, inches-feet	63.21	2,379	57.58	2,309
Survival, percent	45.80	5,179	50.00	5,215
Insect defoliation, index	.63	2,377	.60	2,307

Table 2.--Degrees of freedom (d.f.), mean squares (MS) and significances of differences among families in a one-way analysis of variance of seven traits in two data sets of a northern red oak seedling seed orchard

Source	Data Set: # 1 d.f.	# 1 MS	# 2 d.f	# 2 MS
		HEIGHT		
Among families	218	48.61 ***	217	43.75 ***
Error	2,160	29.83	2,091	27.92
		DBH		
Among families	218	1.81 ***	217	1.77 ***
Error	2,160	1.30	2,091	1.27
		STRAIGHTNESS		
Among families	218	1.19 ***	217	1.19 ***
Error	2,161	.83	2,092	.83
		FORKING		
Among families	218	.93 ***	217	.90 ***
Error	2,160	.68	2,091	.66
		D^2H		
Among families	218	11,550.34 **	217	10,547.66 ***
Error	2,160	8,776.59	2,091	7,734.16
		SURVIVAL		
Among families	219	.68 ***	219	.76 ***
Error	4,977	.23	4,995	.22
		INSECT DEFOLIATION		
Among families	218	.63 ***	217	.59 ***
Error	2,158	.41	2,089	.41

** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level

CONCLUSIONS

The selection intensities in Tables 4 and 5 vary among traits because different families and trees were selected for each trait by independent culling levels. These intensities are lower than they would be for selection for only one trait, and they reflect the compromises needed to achieve selection for more than one trait. The best tree in each plot for every trait is not always selected.

The fact that none of the gains obtained from combined family and single tree selection in Table 6 are large also results from the selection by independent culling levels for seven traits simultaneously. If we had selected only the very best trees in the best families for one trait, the gain for that trait would have been considerably higher. Hence, we believe that the achievement of small positive gains for all seven traits is in itself a worthy accomplishment.

The family heritabilities appear to be small compared to those reported for some other species (Table 3). This probably results from the low effective family size (harmonic mean = nb ~ 10.6 trees per family). With low family sizes, the denominator of the heritability equation becomes larger, thus reducing the value obtained for family heritability.

On the other hand, the single tree heritabilities are fairly normal, and the single tree genetic gains are quite respectable (Table 6), especially when we consider the history of mowing and deer browse problems in this orchard and the fact that selection intensity did not exceed the best one of four trees per plot. We conclude from these gains that phenotypic selection seems to work quite well in a progeny test or seedling seed orchard, in contrast to the conclusion by Farmer (1980) that phenotypic selection in natural stands is not effective for growth traits. However, the small effective family sizes in this study do not permit us to conclude that family selection would not work in a progeny test or seed orchard with larger numbers of trees per family.

This study has at least one major limitation. The results reported here are from only one of 11 locations. Hence, we have no means of accounting for genotype x environment interactions and their potentially negative effects on heritability estimates and genetic gains. In fact, in an initial study of six of the other 10 progeny tests, Farmer (1980) obtained rather low correlations of family means between the Watauga test and the Ohio test ($r = 0.18$) and the Watauga test and the Land-Between-the-Lakes (Tennessee) test ($r = 0.42$).

Nevertheless, the results in this test should be reasonably applicable to any plantations of seedlings from this orchard which might be established in the future on the Watauga District or on any surrounding areas at similar elevations.

A question not directly addressed in this study is the effect of geographic variation. An examination of the 21 rogued families revealed that they broadly represented the geographic seed sources in the whole test. Hence, no geographic effect is apparent.

A primary concern about planting seedlings from this orchard will be that common to regenerating upland oaks in general. Johnson (1986) has described

the use of a medium density shelterwood overstory, which, together with herbicidal control of competing understory vegetation, can result in successful establishment of up to one half of the planted trees two years after the shelterwood is removed.

A serious problem with planting northern red oak is slow initial growth, which averages only one foot per year until the seedlings become established. However, there is an emerging technology using plastic tree shelters which may alleviate this problem. These are available in several forms, and they are being investigated (Tuley 1984). In addition to protecting seedlings from

Table 3.--Family and single tree heritabilities and their standard errors for seven traits in two data sets in a northern red oak seedling seed orchard

	Heritabilities in Data Set:			
	# 1		# 2	
Trait	Family	Single tree	Family	Single tree
Height	0.386 ± 0.044	0.219 ± 0.054	0.362 ± 0.029	0.203 ± 0.054
DBH	.282 ± .039	.139 ± .050	.282 ± .039	.143 ± .049
Straightness	.303 ± .040	.154 ± .049	.303 ± .040	.157 ± .043
Forking	.269 ± .038	.131 ± .047	.267 ± .038	.133 ± .049
D^2H	.240 ± .037	.113 ± .047	.267 ± .038	.133 ± .042
Survival	.662 ± .051	.306 ± .042	.711 ± .057	.375 ± .047
Insect defoliation	.349 ± .042	.189 ± .052	.305 ± .040	.159 ± .051

Table 4.--Selection differentials (SD), genotypic standard deviations (sd_f), and selection intensities (i) of families selected for seven traits in two data sets in a northern red oak seedling seed orchard

	Data Set:					
	# 1			# 2		
Trait	SD	sd_f	i	SD	sd_f	i
Height, feet	0.221	1.315	0.168	0.252	1.223	0.206
DBH, inches	.039	.217	.180	.046	.217	.212
Straightness, index	.029	.182	.159	.036	.184	.196
Forking, index	.018	.152	.118	.018	.151	.119
D^2H, inches-feet	2.582	15.984	.162	2.751	16.304	.169
Survival, percent	.011	.138	.080	.013	.151	.086
Insect defoliation, index	-.009 [1/]	.142	.063	-.011	.130	.085

1/ The choice of this index was such that lower values meant less insect defoliation; hence, negative values mean positive selection differentials.

deer, rodents and understory competition, these translucent or transparent shelters provide a greenhouse effect which seems to enhance height growth.

The results of this test and of recent cultural research suggest a potential for planting genetically improved oak on good sites in the mountains.

Table 5.--Selection differentials (SD), phenotypic standard deviations (sd_w), and selection intensities (i) of single trees selected for six traits in two data sets in a northern red oak seedling seed orchard

Trait	Data Set: # 1 SD	# 1 sd_w	# 1 i	# 2 SD	# 2 sd_w	# 2 i
Height, feet	3.188	5.462	0.584	3.145	5.284	0.595
DBH, inches	.655	1.140	.575	.668	1.127	.593
Straightness, index	.433	.911	.475	.452	.911	.496
Forking, index	.198	.825	.240	.229	.812	.282
D^2H, inches-feet	42.766	93.683	.456	41.809	87.944	.475
Insect defoliation, index	-.169 [1/]	.640	.264	-.163	.640	.255

1/ The choice of this index was such that lower values meant less insect defoliation; hence, negative values mean positive selection differentials.

Table 6.--Percentage genetic gains resulting from family, single-tree, and combined selection for seven traits in two data sets in a northern red oak seedling seed orchard

Trait	Data Set: # 1 Family	# 1 Single tree	# 1 Combined	# 2 Family	# 2 Single tree	# 2 Combined
	Percent	Percent	Percent	Percent	Percent	Percent
Height	0.7	6.0	6.7	0.8	5.7	6.5
DBH	.7	6.0	6.7	.9	6.6	7.4
Straightness	.4	3.0	3.5	.5	3.3	3.7
Forking	.3	1.5	1.8	.3	1.8	2.1
Survival	1.5	--	--	1.8	--	--
D^2H	1.0	7.7	8.6	1.3	9.7	10.9
Insect defoliation	-.5 [1/]	-5.1	-5.5	-.5	-4.3	-4.8

1/ The choice of this index was such that lower values meant less insect defoliation; hence, negative values mean positive genetic gains.

LITERATURE CITED

Becker, W. A. 1984. Manual of Quantitative Genetics, Fourth ed. Academic Enterprises, Pullman, WA. 190 p.

Cech, F. C. 1971. Tree improvement research in oak species. In Oak symposium proceedings, p. 55 - 59. N. E. For. Exp. Sta., Upper Darby, PA.

Core, E. L. 1971. Silvical characteristics of the five upland oaks. In Oak symposium proceedings, p. 19 - 22. N. E. For. Exp. Sta., Upper Darby, PA.

Dixon, W. J. 1983. P7D 9.2 Description of groups (strata) with histograms and analysis of variance. In W. J. Dixon (ed.) BMDP statistical software - 1983 printing with additions, p. 105 - 115. Univ. of California Press, Berkeley.

Farmer, R. E. Progress report. An open-pollinated progeny test in northern red oak (Quercus rubra L.). Division of Land and Forest Resources, Tennessee Valley Authority, Norris, TN. 1980. (Unpublished; 75 p.)

Johnson, P. S., C. D. Dale, K. R. Davidson and J. R. Law. 1986. Planting northern red oak in the Missouri Ozarks: a prescription. North. J. Appl. For. 3: 66-68.

Russell, T. E. 1971. Seeding and planting upland oaks. In Oak symposium proceedings, p. 49 - 54. N. E. For. Exp Sta., Upper Darby, PA.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco, 776 p.

Tuley, G. 1984. Shelters improve the growth of young trees. Aboric. Res. Note 49, DOE Arboric. Adv. and Inform. Service, 4 p.

ANNUAL TOP PRUNING AS A CROWN MANAGEMENT TECHNIQUE IN A YOUNG LOBLOLLY PINE SEED ORCHARD TO REDUCE HEIGHT AND STILL PRODUCE FLOWERS

D. M. Gerwig[1/]

Abstract.--Annual top pruning for five years in Westvaco's loblolly pine breeding clone bank, starting in the 2nd year after grafting, produced flat-topped trees that were 7.9 feet shorter than the unpruned control trees. Flower production per ramet was reduced an average of 30% from 1985 through 1987, but because overall productivity of orchards is low at a young age these losses are tolerable. Top pruning can be used as a crown management tool to produce more uniform orchards and to achieve a height reduction at a young unproductive age to extend the harvestable life of the more productive mature orchard. The flat-topped trees produced by annual top pruning, with all the flowers and cones at one height, may facilitate orchard operations such as supplemental mass pollination.

The use of top pruning as a crown management tool to keep trees shorter to make cones more accessible for collection and also to increase the number of potential flowering branches has been researched for years with inconsistent results. In Australia, top pruning radiata pine (Pinus radiata D. Don) trees either had no effect on cone production or increased cone production (Brown 1971, Matheson and Willcocks 1976, Hand and Griffin 1979). Copes (1973) reported 39% fewer flowers on Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seed orchard trees top pruned annually. Coffen and Bordelon (1981) reported that trees in a western white pine (Pinus monticola Dougl.) breeding arboretum that had forked tops, starting 15 feet up the tree, caused by breakage produced more cones than straight single stem trees. Nienstaedt (1981) top pruned white spruce (Picea glauca (Moench) Voss) in Wisconsin and had 24% more cones on the pruned trees than the controls. Van Buijtenen and Brown (1962), and McLemore (1971) found that top pruning consistently reduced cone yields in loblolly pine (Pinus taeda L.). The studies basically conclude that heights can be controlled with top pruning but that the effect of top pruning on cone production is inconsistent depending on species, age and severity of pruning.

Hand and Griffin (1979) commented on the potential of top pruning in the new orchard concept proposed by Sweet and Krugman (1977). The Sweet/Krugman orchard concept calls for a series of single-clone hedged or top pruned orchards that rely on supplemental mass pollination to produce progeny with a

---

1/ Seed Production Superintendent, Westvaco, Summerville, South Carolina

specific genetic combination. Top-pruned trees of a uniform short height would also be easier to treat with flower initiating or pollen inhibiting chemicals. Applying pollen to flowers or insecticides to conelets and cones all in the top of the tree would be more efficient than treating flowers and cones scattered over a tall tree. Top pruning has not increased flowering in loblolly pine but what Ross and Pharis (1982) say about Douglas-fir can apply to loblolly pine: "Research aimed at determining the optimum tree size to begin treatment and the method and severity of pruning should be given the highest priority..."

METHODS

The study is located in Westvaco's breeding clone bank on the Jericho Unit in Charleston County, South Carolina. The area used for the study contains the plantation selections from Central Woodlands grafted in 1981 in 8-tree clonal rows at a 20- x 20-foot spacing. The selections were made in plantations in north Mississippi and west Tennessee, but monoterpene analysis of two clones indicated the seed may have come from stands in north central Georgia. Four consecutive trees per row were pruned in each row to give a block of pruned trees. Blocking eliminated the problem McLemore (1979) faced when he had to abandon his study after five years because randomly pruned trees were eventually overtopped by the unpruned trees. Hand and Griffin (1979) recommended block pruning as shading reduced the trees ability to respond to topping. Only clones that had at least three pruned and three unpruned ramets were used in the study. Data from 90 control trees and 90 pruned trees from 24 clones were used in this study.

The study deals with annual top pruning in a young developing seed orchard when height growth is high and seed production low. The top pruning treatment evolved with experience and observation. Top pruning began in 1982 during the second growing season after grafting. Total heights were measured before and after top pruning and at the end of the growing season. Total flower counts were made annually starting in the spring of 1984 when the first flowers were produced.

RESULTS AND DISCUSSION

Effects of Pruning on Height

In 1982, a light pruning was made on April 19 after the first flush had set a resting bud. The terminal and any side branches above a target height of 6.3 feet were removed from the pruned treatment trees. The target height was chosen to allow one-half foot of new growth after pruning. Any trees less than the target height were not pruned in order to give these trees a chance to catch up to the taller trees. The objective was to produce a more uniform orchard because small trees in an orchard generally stay small and are later removed because of size rather than genetic potential. Some ramets were grafted near 6.3 feet and these were pruned back to within one-half foot of their individual starting height. Table 1 shows that the first flush grew about the same length for both sets of trees. On the average only one-half foot of new growth was removed, but this is misleading because one-third of the trees were too short to prune. No additional pruning was done in 1982.

Table 1.--Five years of height growth and height reduction data due to annual top pruning

	YEAR									
	1982		1983		1984		1985		1986	
	Control	Pruned	Control	Pruned	Control	Pruned	Control	Pruned	Control	Pruned
	Average Height in Feet									
Starting Height	5.2	5.8	8.4	8.3	11.8	9.8	16.0	11.8	19.6	13.5
Growth of 1st Flush	+1.4	+1.5	+1.4	+1.3	+1.8	+1.8	-	-	-	-
Reduction in Height due to Pruning	0	-0.5	0	-0.9	-	-	-	-	-	-
Growth of 2nd Flush (or 1st plus 2nd)	-	-	+1.9	+1.5	+1.5	+1.5	+2.8	+3.0	+2.9	+3.0
Reduction in Height due to Pruning	-	-	0	-0.6	0	-2.0	0	-2.0	0	-2.0
Additional Growth	+1.8	+1.5	+.1	+.2	+.9	+.7	+.8	+.7	+.5	+.6
Total Growth	3.2	3.0	3.4	3.0	4.2	4.0	3.6	3.7	3.4	3.6
Total Growth after Pruning	3.2	2.5	3.4	1.5	4.2	2.0	3.6	1.7	3.4	1.6
Ending Height	8.4	8.3	11.8	9.8	16.0	11.8	19.6	13.5	23.0	15.1

The light pruning did have a slight effect on average total height as the pruned trees were one-tenth foot shorter than the controls at the end of the growing season.

In 1983, the pruned trees were pruned twice. The first pruning was done on April 19 using a target height of 8.8 feet that again allowed one-half foot of new growth. Twenty percent of the pruned trees had not reached the target height, but pruning was not as conservative as the first year and the target was reached. By the time the second flush set a resting bud the control trees had grown 1.9 feet and the pruned trees had grown 1.5 feet (Table 1). It was hoped that growth of the pruned trees would be much less since the cut ends had to produce buds before resuming new growth. Total heights are measured to the highest leader. What is being measured on the pruned trees are those leaders that were below the pruning height and continued to grow like unpruned trees. The target height for the second pruning was 9.5 feet and the pruned trees were topped on July 29. The control trees grew an average of 3.4 feet for the season. The pruned trees grew an average of 3.0 feet and 1.5 feet of that growth was removed during the two prunings. The pruned trees at 9.8 feet were 2 feet shorter than the control trees after two years of pruning and the crowns were beginning to have a flat-topped appearance.

In 1984, the trees began to produce flowers which influenced the pruning strategy. The first flush in 1984 grew 1.8 feet on both the control and pruned trees (Table 1). Previous top pruning had no effect on present growth. The first flush generally contributes more height growth to the tree than any of the other growth flushes during the year. Therefore, removal of the first flush would reduce height of the tree more than removal of any other flush. However, flowers are born on the terminal end of the first flush and any cutting of the first flush would remove flowers. The objective is to reduce height and increase flowering, not reduce flowering by cutting off flowers. The next best pruning strategy would be to remove the second flush as late as possible to remove as much growth as possible but early enough for the cut terminal to reset buds before flower primordia are produced for the next flower crop in August. By June 29 the second flush had set a resting bud on most trees and elongation of the third flush was just starting on some trees. Two feet of growth were removed which did remove some flowers from the tallest leaders. Again growth after pruning was similar for both the pruned and control trees due to uncut leaders on the pruned trees continuing to grow unchecked (Table 1). After three years the pruned trees were 11.8 feet tall and 4.2 feet shorter than the controls.

Two feet of growth were removed from the pruned trees after the second flush had set a resting bud in 1985 and 1986 (Table 1). Approximately one-half of the annual growth was removed by pruning. At the end of the 1986 growing season the pruned trees were 15.1 feet tall and 7.9 feet shorter than the controls after five years of top pruning.

Annual top pruning of loblolly pine, a species which expresses strong apical dominance, reduces total height (Table 2), but only by the amount of vegetation removed. There does not appear to be any residual effect as Copes (1973) reported in Douglas-fir, that is, pruning one year does not reduce

growth the next year. Table 1 shows that combined growth of the first and second flush the year after pruning on the pruned trees was equal to or greater than growth of the control trees from 1984 through 1986. Growth immediately after pruning was only slightly less on pruned trees because unpruned terminals just below the pruning height continued growth unchecked. In loblolly pine normal growth will resume when pruning is discontinued, only at a lower height; and pruning will have to be done annually to maintain control over height growth.

Table 2.--Average total height of pruned and control trees

Year	Age	Total Height (Ft)	
		Control	Pruned
1982	2	5.2	5.8
1983	3	8.4	8.3
1984	4	11.8	9.8
1985	5	16.0	11.8
1986	6	19.6	13.5
1987	7	23.0	15.1

Top Pruning Effect on Flower Production

Timing of the top pruning treatment is critical in determining the degree of flowering response (Hand and Griffin 1979). Top pruning of loblolly pine in two previous studies was done in the winter or early spring (van Buijtenen and Brown 1962, McLemore 1979). In both cases the cone crop was being removed either as flower primordia in the winter or conelets in the spring. The proper time to top prune loblolly pine is mid summer. Top pruning should be done above the first flush that bears the current conelet crop. The pruning should be done late enough in the growing season to remove the maximum amount of new growth but early enough so that the cut terminals can develop resting buds and develop flower primordia for the next flower crop. In this study pruning was done at the end of June when the second growth flush had set a resting bud and some trees showed beginning elongation of the third flush.

Flower production was clonal as expected (Byram, et al. 1986). Total flower counts per ramet varied from 0 to 427 on control trees and 0 to 309 on pruned trees in 1987. Only four pruned clones produced more flowers than their paired control trees. Basically, those clones that produced many cones or few cones on the control trees consistently produced many cones or few cones on the pruned trees (Figure 1). The pruned trees just produced fewer flowers than the control trees.

Despite pruning at the right time, pruning reduced flower production. Table 3 shows the average number of flowers produced by ramet from 1984 through 1987. In 1984, flower production was reduced nearly 50% but flower production was low. The control trees produced six cones per ramet versus three cones per ramet on the pruned trees. Assuming 97 trees per acre in a production orchard established at a 15- x 30-foot spacing and 250 cones per bushel, this would have been an insignificant loss of one bushel of cones per

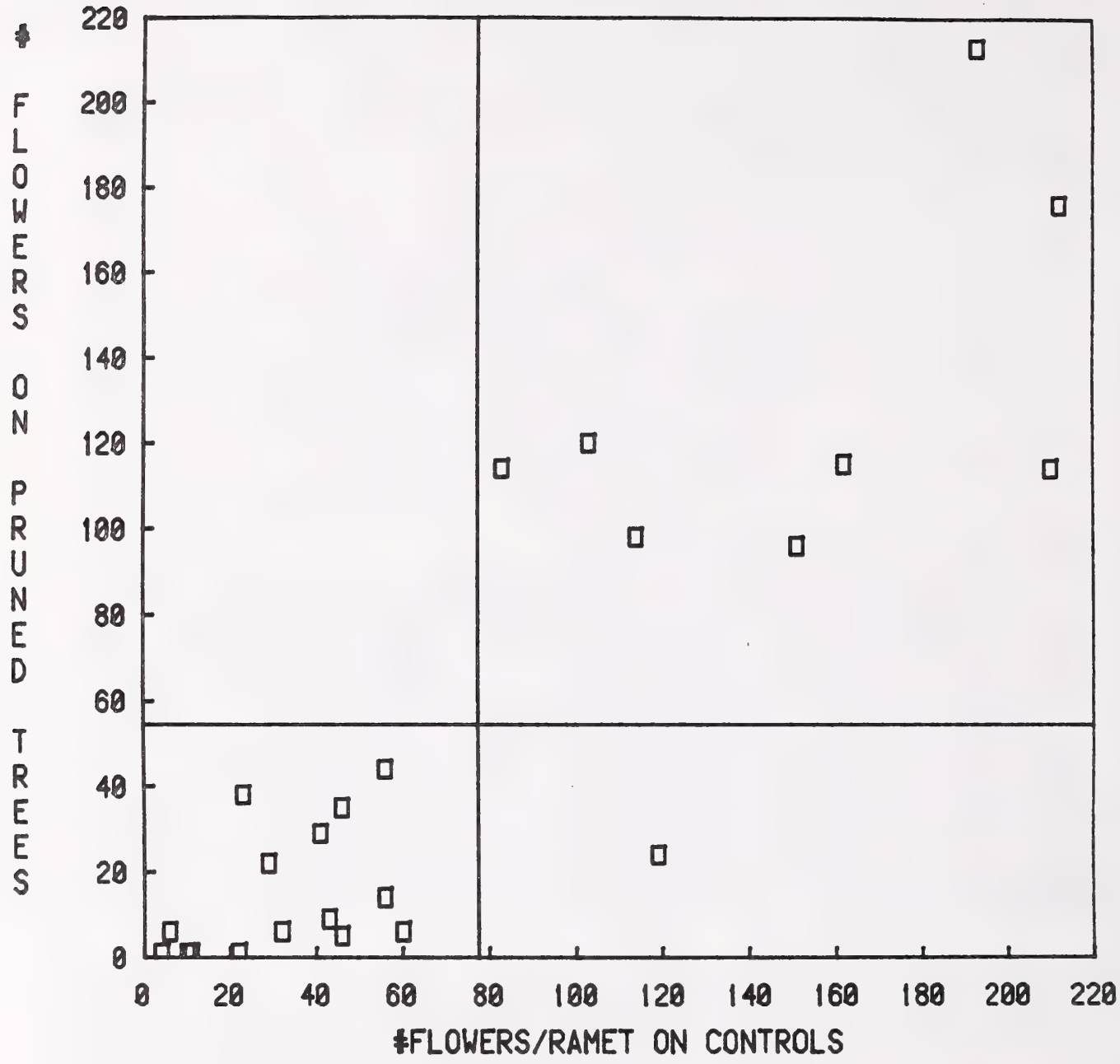


Figure 1.--Flower production per ramet by clone in the top-pruned and control treatments. Each of the 24 squares indicates a clone. The vertical and horizontal reference lines indicate the average number of flowers per ramet on all control ramets (77.5) and all pruned ramets (54.3). Productive clones were productive whether pruned or unpruned. Pruning reduced female flowering on all but four clones.

acre. The 30% loss in flower production in 1987, the best flower crop to date, would have amounted to a loss of 2250 cones per acre or nine bushels of cones per acre if all flowers had developed into cones. The harvest from the pruned trees would have been 21 bushels per acre if all flowers had made cones. If top pruning results in an orchard of uniform height and reduces the height of a mature orchard thereby extending its harvestable life, then some early production losses are tolerable.

Table 3.--Average # flowers per ramet on pruned and control trees

Year	Age	Flower Counts	
		Control	Pruned
1984	4	6.1	3.1
1985	5	37.8	26.8
1986	6	46.2	29.9
1987	7	77.5	54.3

The study will be pruned in 1987 to achieve an overall ten-foot reduction in height from six years of pruning. After 1987, some of the clones will be allowed to resume normal growth in order to determine if the reduction in flowering persists after top pruning stops and to determine if the ten-foot growth differential is maintained throughout the life of the orchard. The other clones will continue to be pruned annually to determine how long a top-pruned orchard can be maintained and what effect continued pruning will have on flower production. Orchard management in the top-pruned orchard would definitely be easier as all trees are the same height and flowers all occur at the same height.

CONCLUSIONS

Annual top pruning of young grafted loblolly pines in mid summer reduces height growth by the amount of growth that is removed. Growth immediately after pruning is not affected as unpruned terminals under the pruned height continue to grow unchecked. Growth the following year is also unaffected by pruning. Pruned trees are going to produce as much new growth as the control trees, but the new growth will start at a lower height due to pruning the previous year.

Annual top pruning reduced flower production an average of 30% in our test even when pruning was done in mid summer.

Top pruning can be used as a crown management techique to control height growth even though flower production is reduced depending on the seed orchard manager's objectives. If the objective is to give small ramets a chance to develop in a newly grafted orchard then top pruning the taller ramets for two or three years will accomplish this with a very minimal loss in production since the orchard is producing very few flowers. If the objective is to reduce height growth at a young age in order to reduce the height of mature trees to extend the harvestable life of an orchard, then pruning for six years after grafting will result in a ten foot reduction in height with a minimum reduction in flower production. If the objective is to produce a

hedged orchard, then annual pruning definitely keeps the flowers at a convenient working height but with a probable loss in flower production.

LITERATURE CITED

Brown, A. G. 1971. Experience in management of a radiata pine seed orchard at Tallaganda State Forest, New South Wales. Aust. For. Res. 5(2): 15-30.

Byram, T. D., W. J. Lowe and J. A. McGriff. 1986. Clonal and annual variation in cone production in loblolly pine orchards. Forest Sci. 32(4):pp 1067-1073.

Coffen, D. O. and M. A. Bordelon. 1981. Stem breakage effect on cone production in Pinus monticola (Dougl.). USDA Forest Service, Res. Note Int-312, 6 p.

Copes, D. L. 1973. Effect of annual leader pruning on cone production and crown development of grafted Douglas-fir. Silvae Genet. 22:167-173.

Hand, F. C. and A. R. Griffin. 1979. Top pruning of Pinus radiata in breeding arboreta. Aust. For. Res. 9:1-7.

Matheson, A. C. and K. W. Willcocks. 1976. Seed yield in a radiata pine seed orchard following pollarding. N.Z. J. For. Sci. 6(1):14-18.

McLemore, B. F. 1979. Top Pruning and bending branches fail to aid collection and production of loblolly cones. Tree Plant. Notes, Spring 1979. pp 27-30.

Nienstaedt, Hans. 1981. Top pruning white spruce seed orchard grafts. Tree Plant. Notes 32:9-13.

Ross, S. D. and R. P. Pharis. 1982. Recent development in enhancement of seed production in conifers: Proc. 18th Can. Tree. Improv. Assoc. pp 27-38.

Sweet, G. B. and S. L. Krugman. 1977. Flowering and seed production problems and a new concept of seed orchards. Proc. 3rd World Consul. Forest Tree Breed. Canberra, Australia, Vol. 2:749-759.

Van Buijtenen, J. P. and C. L. Brown. 1962. The effect of crown pruning on strobili production of loblolly pine. Proc. For. Genet. Workshop, South. For. Tree Improv. Comm. Publ. 22. pp 83 -93.

SUPPLEMENTAL MASS POLLINATION IS FEASIBLE ON AN OPERATIONAL SCALE

F. E. Bridgwater, D. L. Bramlett, and F. R. Matthews[1]

Abstract.--A commercially available pole duster with a compressed-air tank for pollen delivery was used to pilot-test supplemental pollination to increase genetic gains in loblolly pine on an operational scale. Two operators working together required an average of 19 minutes to treat each ramet, which were approximately 50 feet tall with an average of 174 clusters of female strobili. Proper timing of applications was critical; those made after peak pollen shed in the seed orchard were not effective.

Additional keywords: Pinus taeda, fusiform rust.

Supplemental mass pollination (SMP) has been shown to be effective for increasing genetic gains from loblolly pine (Pinus taeda L.) seed orchards (Bridgwater and Williams 1983). They showed that a single, properly timed application of supplemental pollen to strobili not isolated from windborne pollen resulted in an average of 86% selected pollen grains in ovules. However, their study employed destructive sampling to determine the proportion of dyed pollen in ovules and did not directly measure the proportion of seeds arising from supplemental pollen. Furthermore, their experiment was done on a scale much smaller than would be required for an operational SMP program where timing and methods for application would be more difficult. The trial reported here was conducted to test the feasibility of SMP to increase genetic gains using methods and equipment that would be required in an operational program.

A second objective was to determine the time required to do SMP to aid in planning operational SMP programs.

METHODS

One way to increase genetic gains from SMP is to capitalize on good specific combinations. If specific crosses among orchard parents are known to be better in some respect than wind-pollinated seeds from the same parents, then repeating those favorable crosses with SMP will increase the genetic potential of their offspring to the extent that it is successful.

1/ Research Geneticist, Project Leader, and Plant Pathologist, respectively, USDA Forest Service, Southeastern Forest Experiment Station. The authors wish to thank Billy Arnold, the Georgia-Kraft Co., Weyerhaeuser Co., and the North Carolina State University-Industry Tree Improvement Cooperative for their assistance in conducting this study.

Five good specific crosses among seven seed orchard parents were identified in the Briarpatch seed orchard of the Georgia-Kraft Company in Putnam County, Georgia. These crosses were chosen primarily for resistance to fusiform rust (Cronartium quercuum [Berk.] Miyabe ex Shirae f. sp. fusiforme), and secondarily for good growth rates. Performance was determined by examining performance levels, which are relative scores for all the clones and specific crosses among them (Hatcher et al. 1981). None of the five crosses had been tested in fewer than four progeny tests and all had been evaluated for 8 years in at least one test.

The success of SMP was estimated by comparing the percentage of rust-free seedlings arising from controlled pollinations, SMP, and wind pollinations on ramets of the same genotypes. The difference between the percentages of rust-free seedlings from the controlled-pollinated (CP) and wind-pollinated (WP) seedlings represented the potential for improvement, and the difference between percentages of rust-free seedlings from SMP ramets and from SMP ramets and WP ramets measured the success of SMP. Thus, if CP seedlings were 31% rust free, SMP seedlings were 24% rust free, and WP seedlings 21% rust free, the SMP success would be 30%. The percentage of rust-free seedlings was increased 3% with SMP while the potential improvement was 10% with CP.

At least 1.5 liters of pollen were collected and processed for storage from each of three pollen parents during 1983. Pollen was dried for 24 hours at 39°C to 5% to 6% moisture content, and frozen in quart jars at -20°C until application during spring, 1984. In vitro pollen viability was determined 1 month before pollen was applied and was found to be 72%, 80%, and 86% for the three pollen parents.

Control pollinations were made by using standard procedures for the southern pines on one ramet of each clone. A second ramet received SMP when a majority of female strobili reached stages 4L-5 (Bramlett and O'Gwynn 1981). Supplemental pollen was applied with a commercially available pole duster illustrated and described in Figure 1. The pole duster and compressed-air tank complete with fittings cost less than $150 when purchased in 1983. A third ramet of each clone far enough removed from the SMP ramet to avoid the possibility of accidental pollination was tagged for the collection of wind-pollinated seeds.

A crew of two applied the supplemental pollen from a bucket truck. One crew member operated the bucket controls and kept track of the progress of pollinations to ensure that most, if not all, strobili received supplemental pollen. The second crew member applied 1 to 1.5 ml of pollen to each cluster of female strobili by simply lowering the windscreen over each cluster and applying two short bursts of compressed air. The pole duster had previously been calibrated by trial and error to apply 0.5 to 0.75 ml pollen per burst of compressed air. This amount of pollen was judged to be sufficient for pollination by applying different amounts of dyed pollen with the pole duster and subsequently dissecting the female strobili to determine pollination effectiveness.

Cones were harvested in the fall of 1985, and seeds were extracted and processed for sowing in the spring of 1986. Both a field study and an

Figure 1. Illustration of the pole duster used in this trial. The pole duster is a 6-foot section of 1/2-inch diameter aluminum tubing with a pollen bottle installed in-line. A 6-inch diameter aluminum funnel served as a windscreen which is placed over a cluster of strobili. A compressed-air supply was fitted to the base of the pollinator where a thumb-operated level controlled the air supply and, thus, pollen emission. An emitter was devised to place over the 1/8-inch copper tubing in the windscreen so that pollen was forced against the sides of the windscreen and onto the strobili from all sides.

artificial inoculation test in a greenhouse were initiated in the spring of 1986. Unfortunately, the field test failed due to a drought, thus only the results of the greenhouse study are reported here.

The inoculum source was aeciospores of the fusiform rust fungus collected in 1974 from loblolly pine galls in Clark County, Georgia. This bulk spore collection was processed and stored according to the procedures outlined by Roncadori and Matthews (1966). Basidiospores used in the pine inoculations were produced by infecting seedlings of northern red oak (Quercus rubra L.) with the aeciospores. The basidiospores were harvested from the oak leaves and used in a concentrated basidiospore spray (Matthews and Rowan 1972). Pine seeds were germinated, and immediately after emergence were transplanted into flats in lots of 20 each. At 4 weeks of age, the seedlings were passed on a conveyor belt under an aqueous spray of basidiospores. The inoculum contained 50 M spores/ml and was dispersed in 8 ml aliquots on each flat of seedlings as it passed under the spray nozzle. In vitro germination of basidiospores immediately prior to inoculation was 85%.

The inoculum was applied to 6 flats of 20 seedlings for most seed lots, but some lots had fewer germinants and, thus, a reduced number of seedlings were available for inoculation. Immediately after inoculation, the seedlings were placed in a mist chamber and held at 20°C for 24 hours. They were then grown in the greenhouse for 6 months before being examined for

infection. Seedlings with active rust galls were used to determine the percentage of infected seedlings. The numbers of seedlings used to determine the percentages of rust-free seedlings ranged from 26 to 120, and averaged 107. Seeds from a susceptible check seed lot, #4666-4 from Houston County, Georgia, were used to evaluate inoculation results.

Percentages of rust-free seedlings were analyzed by standard analysis of variance procedures. Both percentages and their transformed values (square root transformation) yielded the same results, therefore percentages are reported here.

RESULTS AND DISCUSSION

SMP Success

SMP was successful when done before peak pollen flight occurred in the seed orchard (Figure 2). The percentage success was 144% and 30% for crosses 556x521 and 110x556, respectively. The percentage of rust-free seedlings in the SMP treatment exceeded the percentage in the CP treatment for the cross 556x521. This probably occurred as the result of sampling errors, which were large due to the small numbers of seedlings in the study. In any event, the differences between CP and SMP treatments were not significantly different at the alpha = 0.10 level. Since there were no significant interactions between treatments and the two crosses made before pollen was shed in the seed orchard, another analysis of variance was made for their pooled data. The average percentage of rust-free seedlings was 26% for SMP and 28% for the CP crosses on the two females receiving SMP before pollen was shed in the seed orchard. That is, the estimate of the average percentage success was 80%. However, the coefficient of variation for the percentage of rust-free seedlings was 44%. Thus, SMP appears to be feasible when operational procedures are simulated, but the level of success is still in question.

SMP was not successful on the three females that received treatment after peak pollen shed in the seed orchard (Figure 3). Strobili on these female parents were observed to be in stages 4-4L, the stages just preceding stage 5, or full receptivity, on the morning of the day of peak pollen shed in the seed orchard. If this had truly been an operational program, SMP should have been done at that time in anticipation of pollen shed. Delaying SMP in this trial demonstrated the ineffectiveness of applications after peak pollen shed in the orchard.

There were significant interactions among these three females and treatments, therefore the response of each female was considered individually. Crosses 182x521 and 114x521, respectively, received SMP 24 and 48 hours after the peak of seed orchard pollen shed began. There were no significant differences among the three treatments for 182x521, but for 114x521, the percentage of rust-free seedlings for the CP treatments was significantly less (10%) than either SMP or WP treatments (22% and 24%, respectively). The expected mid-parent value based on wind-pollinated values for this cross was 21%, which suggests that there was negative specific combining ability for resistance to fusiform rust in this experiment. In that case, the proportionate reduction in the percentage of rust-free seedlings with

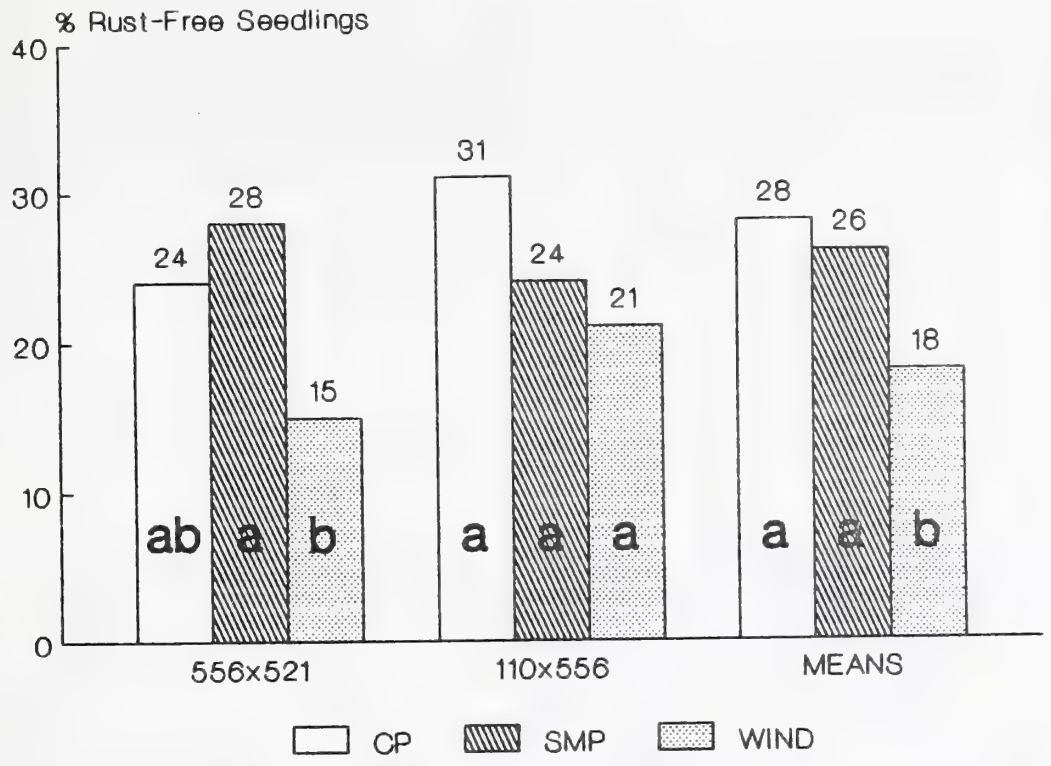


Figure 2. Percentages of rust-free seedlings from controlled-pollinated (CP), supplemental mass pollinated (SMP), and wind-pollinated females when SMP was done before the peak of seed orchard pollen shed occurred. Treatment bars annotated with the same lower case letter were not significantly different at P = 90%.

controlled pollination compared with wind pollination would indicate the level of success from SMP. However, the small difference measured was not significant. The cross 1027x1542 had the greatest difference between the CP and WP treatments and would have offered the best opportunity to quantify the success of SMP. However, there was no significant difference between the percentages of rust-free seedlings with SMP applied after peak seed orchard pollen shed and wind pollination.

Time Required for SMP

The time required to do SMP treatments was surprisingly short and predictable. The numbers of clusters of strobili per ramet and the times required to set up the bucket truck and accomplish the SMP application are given in Table 1.

The linear regression of minutes required for SMP on numbers of clusters of female strobili was Minutes = 10.7 + 0.048 (clusters of strobili) with a standard error of 1.2 minutes. This prediction equation will facilitate planning operational SMP programs.

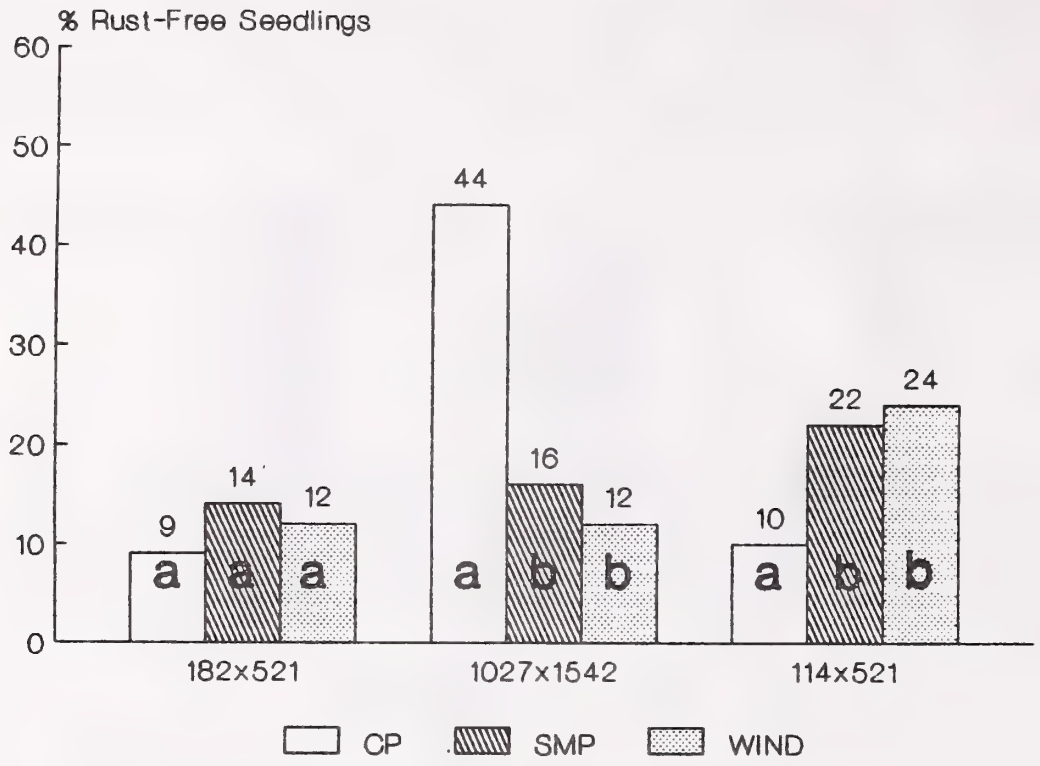


Figure 3. Percentages of rust-free seedlings from controlled-pollinated (CP), supplemental mass pollinated (SMP), and wind-pollinated females when SMP was done after the peak of seed orchard pollen shed occurred. Treatment bars with the same lower case letter were not significantly different at P = 90%.

Table 1.--Number of clusters of strobili and time required per ramet for SMP

Ramet number	Clusters of strobili (No.)	Time for SMP (min.)
1	153	18
2	189	22
3	162	18
4	214	20
5	238	22
6	93	15
Mean	174	19

CONCLUSIONS

Supplemental mass pollination is feasible on an operational scale. Supplemental pollen must be applied to receptive female strobili before peak pollen shed in the seed orchard to ensure success. Supplemental pollen applied after peak seed orchard pollen shed was not effective.

The number of man-hours and the equipment required for SMP put such programs within the reach of every operator who wishes to implement them.

LITERATURE CITED

Bramlett, D. L. and C. H. O'Gwynn. 1981. Controlled pollination. In: Pollen Management Handbook, E. C. Franklin, ed., USDA Forest Service, Agri. Handb. 587. p. 44-51.

Bridgwater, F. E. and C. G. Williams. 1983. Feasibility of supplemental mass pollination to increase genetic gains from seed orchards. In: Proc., 17th Southern Forest Tree Improvement Conf., Athens, Georgia, June 7-9, 1983. National Technical Information Service, Springfield, VA. p. 78-83.

Hatcher, A. V., F. E. Bridgwater and R. J. Weir. 1981. Performance level--a standarized score for progengy test performance. Silvae Genet. 30(6):184-197.

Matthews, F. R. and S. J. Rowan. 1972. An improved method for large-scale inoculations of pine and oak with Cronartium fusiforme. Plant Dis. Rep. 56:931-934.

Roncadori, R. W. and F. R. Matthews. 1966. Storage and germination of aeciospores of Cronartium fusiforme. Phytopathology 56:1328-1329.

CLONAL VARIATION OF TANNIN AND PHENOLIC CONCENTRATIONS OF CONES FROM A SELECTED POPULATION OF LOBLOLLY PINE

T. N. Trembath[1]/ and G. R. Askew[2]/

Abstract.--Cone samples were collected from 19 clones in a chemically treated loblolly pine seed orchard every 2 months between March 1985 and May 1986. Tannin and phenolic concentrations of the cones was quantified by chemical analysis to examine the clonal and seasonal variation and to determine if variability in tannin and phenolic concentration was related to insect susceptibility. Both tannin concentration and phenolic concentration varied throughout the cones lifecycle. Tannin concentration consistently peaked for all 14 clones at age 10 mo. Total phenolics were not found to be as consistent for all clones as were the tannins. Both tannin concentration and phenolic concentration had significant variation associated with cone age. No single clone had a consistently higher or lower overall mean tannin or mean phenolic concentration than any other clone and no relationship was found between mean tannin concentration or mean phenolic concentration of cones and seed bug and coneworm infestation levels.

Coneworm and seed bug damage is a major problem in loblolly pine seed orchards. Cone and seed damage estimates as high as 90% have been found in some untreated southern pine seed orchards (Ebel et al. 1981), with Dioryctria amatella, D. disclusa, D. merkeli, Leptoglossus corculus and Tetyra bipunctata being the primary damaging insects. Coneworms, Dioryctria spp., destroy the entire cone by tunnelling and feeding within the cones. Seed bugs, Leptoglossus corculus and Tetyra bipunctata destroy individual seeds by puncturing the conescales with needle-like mouthparts and extracting nutrients from the seeds.

Application of insecticides is the current control practice in seed orchards, but because of the high monetary cost and potential adverse environmental effects of pesticides, there has been increased interest in exploiting genotypic variation in coneworm and seed bug susceptibility to reduce losses from these pests. Askew et al. (1985) noted significant genetic variation in coneworm and seed bug attacks among loblolly pine clones.

Many factors, including the levels of defensive plant chemicals, may determine the reduced susceptibility of some lobolly pine trees to pest attack. Schultz stated (in Maugh 1982) that chemicals produced by plants may be more important than any other single factor in controlling insects in nature and phenolics and tannins have been shown to be related to the level of herbivore damage in some plant species (Feeny 1976).

---

[1]/ Graduate Research Assistant, Clemson University, Clemson, South Carolina.

[2]/ Associate Professor of Forestry, Belle W. Baruch Forest Science Institute of Clemson University, P. O. Box 596, Georgetown, South Carolina 29442.

Many studies of phenolics of coniferous tree species have been completed, but nearly all the work has been with wood, bark, or foliage (Hillis 1962, Forrest 1975, Hergert 1960). Little information has been reported about phenolic compounds in conifer cones. Hillis (1962) stated that concentrations of various polyphenols in different tissues of the same tree can vary considerably and that once formed, translocation of these substances is improbable. Information about the occurrence and variation of phenolic compounds in cones is needed in order to learn more about the relationship of phenolic compounds with cone and seed insects. In this paper we will discuss the variations of tannin and phenolic concentration of cones among clones in a loblolly pine seed orchard and measurements of tannin and phenolic concentrations as the cones age. We will also examine the variability in tannin and phenolic concentration of cones as it is related to susceptibility to insect attack.

STUDY AREA

Loblolly pine cone samples were collected from 19 clones at a coastal seed orchard located near Georgetown, South Carolina. The orchard is 10.52 ha in size, with a 4.57 m by 9.14 m original tree spacing. An average spacing of 9.14 m was achieved through orchard roguing, with some spacing being as wide as 18.29 m by 9.14 m. The trees were 22 years old at time of initial sampling and had an average height of 19.81 m. The orchard was chemically treated from the ground with a mist blower on a monthly basis from April through August, using Guthion 2S for seed bug control and Pydrin for coneworm control.

Cone Collection

Two ramets from each of the 19 clones in the orchard were selected for sampling throughout the study. Ramets were selected such that several trees could be sampled without moving the equipment. Due to orchard roguing and unavailability of cones from some trees, the number of ramets per clone and the number of clones varied throughout the study. Whenever possible, a new ramet was selected for those that could no longer be sampled.

We attempted to collect 4 cones per ramet every 2 months, beginning May 1985 when the cones were 2 months old and ending in March 1986, but the actual number of cones collected in a sampling month varied depending on cone availability. Cones of two different ages were collected during the sample months of May 1985 and July 1985. In May 1985 2 month old and 14 month old cones were collected, and in July 1985 4 month old and 16 month old cones were collected. By simultaneously sampling both young and old cones, all age groups were collected in one year rather than across an 18 month period. The 6 month old cones were not collected due to the unavailability of collection equipment in September 1985. Cones were randomly selected from each tree without regard to position. After collection, the cones were transported to the lab in a portable cooler and then stored in a walk-in cooler until they were processed.

Extractions

Phenolic compound extraction was conducted on a cross-section sample of individual cones or on the entire cone if they were small enough to be easily handled. Samples were pooled by ramets, chopped, frozen in liquid nitrogen

and then ground by hand using a mortar and pestle. The mature 18 month old cones that were collected in September 1985 were too woody to be ground by the liquid nitrogen method, so they were pooled together by ramets, chopped, and then ground in a Wiley Mill to pass through a 1 mm mesh screen.

Extraction of the tannin and phenolic compounds was accomplished by placing 1.25 grams of the ground tissue (or less if the cones were small) from each pooled ramet sample into 50 ml of 80% methanol maintained at 80°C for ten minutes (Buchsbaum et al. 1984). Two sequential extractions were completed if there was sufficient tissue. After extraction, all samples were filtered by gravity through No. 1 Whatman filter paper and the sequential extractions were pooled.

Extraction of all cone samples for a collection date was completed within 4 to 6 weeks after cone collection. Two 8 ml vials of the pooled extractions were stored in a refrigerator until they could be analyzed. All analyses were completed within 4 to 6 weeks after extraction.

Tannin Analysis

Tannin content of the extract was determined as follows: 1) 100 ml of fresh steer blood was collected and immediately lysed by the addition of 50 ml of cold distilled water. The mixture was returned on ice to the laboratory, and then centrifuged to produce a solution that was free of cell membranes. A 5% blood solution for use in the analysis was then prepared in distilled water from this stock. 2) Tannic acid standards of 0, 500, 750, 1000, 1250, 1500, 1750, and 2000 ppm were prepared in distilled water and used for the Hemoglobin test. Three ml of tannin extracts or standard solutions were added to 3 ml of 5% blood in a centrifuge tube, vortexed until the solution was homogenous (about 10 seconds), and then centrifuged at 9000 rpm at 21°C for 30 minutes. Absorbances of the solutions were determined at 578 nm on a SPECTRONIC 88 spectrophotometer calibrated to an absorbance of 2.0 with a blood blank. Tannic acid equivalents (TAE) were determined for each sample from a plotted curve of the tannic acid standards. TAE was defined as the ratio of the sample concentration predicted from the standard curve to the actual dry weight concentration of the extracted material (Bate-Smith 1973).

Phenolic Analysis

Total phenolic content of the extracts was determined by using the Folin-Denis procedure as described by Swain and Hillis (1959). Aliquots of the extracts were diluted with distilled water to 1%. Tannic acid standards of 0, 20, 40, 60, 80, and 100 ppm were used. Equal volumes (usually 3 ml) of tannin extracts and Folin-Denis reagent were combined in a centrifuge tube. After 3 minutes, an equal volume of 2N sodium bicarbonate solution was added as a fixative. Tubes were vortexed to homogenize the liquids after a 1 hour incubation at room temperature. Sample absorbances were determined at 725 nm on a SPECTRONIC 88 spectrophotometer calibrated to an absorbance of 2.0 with a 100 ppm standard.

Seed Analysis

Ten mature cones free of coneworm damage were collected from each of the clones in October 1985. Cones were dried at the Southeastern Forest

Experiment Station Forestry Sciences Lab in Athens, Georgia. In January of 1986 the seeds were extracted from the dried cones and x-ray analysis was used to determine the degree of seed bug damage for each clone. The seed bug damage was categorized as percent full, percent empty, percent abort (second year abortion of ovules early in the second growing season), percent seed bug (damage to the seeds late in the second growing season), percent seedworm (Laspeyresia spp.), and percent fungi.

Cone Analysis

All coneworm infested cones were separated from the 1985 bulk collection of each ramet and the species of coneworm was identified. The total number of cones harvested for each ramet was not determined, so the proportion of infested cones for each clone was calculated based on a bushel estimate from the previous three years.

Data Analysis

Analysis of variance was used to evaluate the effects of age on the average tannin and phenolic concentration of all clones and to examine the interactions of age by clone and age by ramet within clone, with Type I error set at 0.05. Correlation analysis was used to examine the relationship of tannin and phenolic concentrations at each age with the seed but damage levels for each clone. Only those clones that had tannin and phenolic data for all ages, excluding 6 and 18 months, were analyzed.

RESULTS AND DISCUSSION

Seasonal Variation of Tannin and Phenolic Concentration

Tannin concentrations and phenolic concentrations (Table I) varied throughout the life cycle of loblolly cones. Tannin concentration consistently peaked for all 14 clones in January samples (Age 10 mo.), while July samples (Age 16 mo.) showed the lowest tannin concentration in all but one clone. Total phenolics were not as consistent for all clones as were the tannins. Nine of the 14 clones peaked in mean phenolic concentration in January samples (Age 10 mo.), two clones peaked in November samples (Age 8 mo.), 2 clones peaked in July samples (Age 16 mo.), and 1 clone peaked in March samples (Age 12 mo.). Age of cones at time of lowest mean phenolic concentration was more variable among all clones than peak concentration. Six clones had the lowest mean phenolic concentration in May samples (Age 4 mo.), 2 clones in July samples (Age 16 mo.), and 1 clone in May samples (Age 2 mo.).

Analysis of variance revealed an age effect for both tannin concentration and phenolic concentration and a clonal effect for phenolic concentration alone (Table II). These results were as expected due to the obvious peak in both tannin and phenolic concentrations at age 10 mo. The clonal effect for phenolics was not of interest by itself, but will be discussed later in connection with the cone and seed insect data.

The large reduction in phenolic concentration from January samples (Age 10 mo.). to May samples (Age 14 mo.) may be attributed to the depletion of starch caused by the vegetative flush of growth in the spring. Phenolic concentration rapidly increased from May samples (Age 14 mo.) to July samples

(Age 16 mo.). This increase may be explained by the results of a study by Chung and Barnes (1980) who found that the time of rapid increase in the fraction of photosynthate in loblolly pine allocated to protection constituents (phenolics) late in the growing season seemed to be correlated with the termination of axis and needle elongation.

Table I.--Mean tannin and phenolic concentrations of cones for each age.

Age (Mo.)	Mean Tannin concentration (ppm TAE)	Std Error	Mean Phenolic concentration (ppm TAE)	Std Error
2	1432	23.16	48	2.54
4	1258	14.03	48	3.70
8	1259	13.23	52	2.72
10*	1832	36.99	67	2.25
12	1450	16.81	59	2.27
14	1417	15.37	43	2.34
16	970	36.65	53	3.53

*Indicates peak age for tannin and phenolic concentrations

Table II.--Analysis of variance for tannin and phenolic concentrations.

		Tannin		Phenolic	
Source	df	MS	F	MS	F
Clone	13	32217.62	1.46	665.04	3.07*
Ramet (Clone)	14	22022.11	1.44	216.49	0.93
Age	6	1919211.80	140.91*	1776.82	12.66*
Clone x Age	78	13620.56	0.89	140.40	0.60
Error	84	15298.39		232.30	

*Calculated F exceeds tabulated F with a Type I error rate of 0.05

Correlation of Mean Tannin and Mean Phenolic Concentration of Cones To Coneworm and Seedbug Infestation Levels

No relationship was found between mean tannin concentration or mean phenolic concentration (Table III) of cone tissue and percent of dead cones attributed to attack by coneworms. Specifically, per bushel average of percent small dead cones attributed to attack by Diorctria merkeli in late (May samples) was compared to the tannin and phenolic concentrations of cones at ages 14 mo. and 16 mo. for these clones. No relationship of low percent

dead cones to high mean tannin concentration or mean phenolic concentration was apparent. The same was true for high percent dead cones and low mean tannin concentration or mean phenolic concentration.

A per bushel average was also determined for the percentage of large dead cones attributed to attack by Dioryctria amatella in mid to late summer and compared with mean tannin concentration and mean phenolic concentration at age 16 mo. (July samples). Again, no relationship was found between high or low percent dead cones and low or high mean tannin concentration or mean phenolic concentration. For example, Clone 11 (ramet 1) had the same per bushel average (7%) of small dead cones (May attack) as large dead cones (July attack), but the tannin concentration of 1359.3 ppm at age 14 mo (May sample) was much higher than the tannin concentration of 948.7 ppm at age 16 mo (July sample). Ramet 2 of the same clone had a 79% per bushel average of small dead cones (May attack) and a 51% per bushel average of large dead cones (July attack). However, the tannin concentration of 1384.9 ppm at age 14 mo (May sample) was much higher than the tannin concentration of 954.7 ppm at age 16 mo (July samples).

Table III.--Per bushel average of percent small dead cones attributed to attack by Dioryctria merkeli in late May to early June (Age 14 mo.) and percent large dead cones attributed to attack by Dioryctria amatella in mid to late summer (Age 16 mo.) with corresponding mean tannin and phenolic concentrations.

Clone	Small Dead Cones	Large Dead Cones	Tannin 14	Tannin 16	Phenol 14	Phenol 16
	%	%	ppm	ppm	ppm	ppm
1	2.7	5.7	1400	1231	34	50
2	11.0	23.0	1533	924	48	65
5	5.0	4.0	1425	826	45	57
6	5.0	6.5	1455	1073	42	65
7	13.0	14.0	1501	1056	48	63
9	2.0	1.3	1444	846	56	57
10	3.5	5.0	1419	762	44	42
11	42.0	29.0	1372	970	41	54
Ramet 1	7.0	7.0	1359	985	38	64
Ramet 2	79.0	51.0	1385	955	44	43
14	2.5	6.0	1268	1111	39	41
15	6.0	21.0	1378	870	47	52

A significant correlation was found between mean phenolic concentration at age 16 mo. (July samples) and percentage of aborted, full, and empty seeds ($r = -.54$, $.76$, and $-.74$, respectively). Reasons for this correlation are unapparent at this time. A correlation earlier in the growing season would be expected because the percent abort figure represented the percentage of aborted ovules early in the second growing season. Also, no correlation was

found between percent seed bug infestation and mean phenolic concentration at age 16 mo. (July samples), which would be late in the second growing season.

There are several possible explanations for not finding a relationship of mean tannin and mean phenolic concentration to seed bug and coneworm infestation levels. Most importantly, the orchard was sprayed from the ground using a mist blower from April through August with Guthion 2S for seed bug control and Pydrin for coneworm control. Expected effectiveness of this treatment is 80 to 90 percent. Because some trees in the orchard may not have been as effectively sprayed as others due to differing total heights and crown depths, any differences in infestation levels among clones found in this study may not be the same if the entire orchard was untreated or sprayed at a consistent level. However, even if the orchard was sprayed at a consistent level, differences in infestation levels would still be expected. Another explanation may be that quality of tannins and phenolics, rather than quantity, is responsible for susceptibility or resistance to insect attacks. In trees with low tannin levels, phenolic levels, and low infestation levels, a specific phenolic compound may be present which is responsible for the tree's decreased susceptibility. The opposite may be true for trees with high tannin and phenolic concentrations with high infestation levels. Some trees that had a high infestation level also had high concentrations of mean tannins and mean phenolics. If these trees had lower mean tannin and mean phenolic concentrations the infestation level may have been even greater. One last possibility may be that once a tree is attacked it will increase production of phenolic compounds as a response to the attack. Schultz and Baldwin (1982) reported that mechanically damaged poplar (Populus x euromericana) ramets and sugar maple (Acer saccharum Marsh) seedlings showed increased concentration and rates of synthesis of phenolic compounds.

Drought or other growth stress can influence phenolic production (Forrest 1975). Because of the high energy cost associated with carbohydrates being used for phenolic production rather than for growth, a stressed tree would probably produce less phenolic compounds than would a healthy, vigorous tree. Available carbohydrates would be allocated to growth and foliage production rather than secondary plant substances (Hillis 1968). For this reason, we feel that cones would be more susceptible to insect attack during stress years. However, a more vigorous tree would generally produce more cones than would a stressed tree, thus there would be more available infestation sites on a more vigorous tree.

SUMMARY AND CONCLUSIONS

Tannin and phenolic concentrations varied throughout the lifecycle of loblolly cones. Tannin concentration was highest for all clones at age 10 mo. and lowest for all but 1 clone at age 16 mo.

The pattern of phenolic concentration was less consistent for all clones than was the tannin concentration. Most clones peaked in phenolic concentration at age 10 mo.; however, the age of cones at time of lowest phenolic concentration varied among clones. Depletion of starch caused by the vegetative flush of growth in the spring may be responsible for the decrease in phenolic concentration in May.

Overall analysis of variance of mean tannin concentration and mean phenolic concentration of cones revealed age to be a significant factor. Variation of tannin and phenolic concentration of cones among ramets within clones and interactions of clone by age were not significant, but a significant level of clonal variation was found for mean phenolic concentration of cones.

No relationship was found between mean tannin concentration or mean phenolic concentration of cones and seed bug and coneworm infestation levels. Possible reasons for the lack of correlation may be non-uniform chemical treatment of the orchard studied or that quality of tannins and phenolics, rather than quantity, was responsible for susceptibility or resistance to insect attacks.

Further research on tannin and phenolic concentration of loblolly pine cones should include an examination of the relationships of crown position of cones and their tannin and phenolic concentrations. Cones from orchards not treated with insecticides need to be examined and the phenolic concentration of seeds, cone axes, and conescales should be studied individually to better understand the relationship of tannin and phenolic concentration to insect resistance or susceptibility. We feel that the most valuable information to be gained from future research will be the delineation of the specific phenolic compounds that are present in the cones throughout their lifecycle and the correlation between these specific phenolics and insect resistance or susceptibility.

LITERATURE CITED

Askew, G. R., R. L. Hedden, and G. L. DeBarr. 1985. Clonal variation in susceptibility to coneworm (Dioryctria spp.) in young loblolly pine seed orchards. For. Sci. Vol. 31, No. 3. pp. 794-798.

Bate-Smith, E. C. 1973. Haemanalysis of tannins: the concept of relative astringency. Phytochemistry 12: 907-912.

Buchsbaum, R., I. Valiela, and T. Swain. 1984. The role of phenolic compounds and other plant constituents in feeding of Canada geese in a coastal marsh. Oecolgia 63: 343-349.

Chung, H-H., and R. L. Barnes. 1980. Photosynthate allocation in Pinus taeda. II. Seasonal aspects of photosynthate allocation to different biochemical fractions in shoots. Can. J. For. Res. 10: 338-347.

Ebel, B. H., T. H. Flavel, L. E. Drake, H. O. Yates, III, and G. L. DeBarr. 1981. Seed and cone insects of southern pines. U.S. Dept. Agric. For. Ser. Gen. Tech. Rep. SE-8, 40p. Southeast For. Exp. Stn., Asheville, N.C., and Southeast Area, State and Priv. For., Atlanta, Ga.

Feeny, P. P. 1976. Plant apparency and chemical defense. Recent Ad. Phytochemistry. 10: 1-40.

Forrest, G. I. 1975. Polyphenol variation in sitka spruce. Can. J. For. Res. 5: 26-37.

Hergert, H. L. 1960. Chemical composition of tannins and polyphenols. Forest Prod. J. 10: 610-617.

Hillis, W. E. 1962. The distribution and formation of polyphenols within the tree. pp. 59-131 in W. E. Hillis (ed.) Wood extractives and their significance to the pulp and paper industries. Academic Press, New York. 1962. 513 pp.

Hillis, W. E. 1968. Chemical apsects of heartwood formation. Wood Sci. Technol. 2, 241-259.

Maugh, T. H. 1982. Exploring plants resistance to insects. Science 26: 722-723.

Schultz, J. C. and I. T. Baldwin. 1982. Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217: 149-150.

Swain, T, and W. E. Hillis. 1959. The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63-68.

THE EFFECTS OF USING POLLEN CONTAMINATED WITH CONIDIA OF FUSARIUM MONILIFORME VAR. SUBGLUTINANS ON CONTROL-POLLINATED STROBILI OF SLASH PINE

T. Miller,[1/] G. M. Blakeslee,[2/] D. L. Bramlett[3/] and F. R. Matthews[4/]

Abstract.--Strobili of slash pine were inoculated at the time of pollen receptivity with a mixture of pollen and conidia of Fusarium moniliforme var. subglutinans (FMS). FMS was subsequently isolated from apophysis, ovule, and axis tissues of relatively few sampled strobili. Mean strobilus losses through 10 months were greater in FMS-inoculated treatments than in treatments with pollen alone or wind-pollinated controls. FMS was not isolated from living conelets sampled at 10 months, and the fungus was recovered from only 1 of 64 conelets collected after 11 months. The pathogen was not cultured from seeds of cones that survived to maturity. Under the conditions of this research, seed yields and seed infections did not appear to be influenced by exposure of strobili to conidia of FMS at the time of pollen receptivity.

Additional keywords: Pinus elliottii var. elliottii, pitch canker, inoculations, seed damage, seed orchards.

In the mid-1970's, an investigation of the causes of poor seed germination of slash pine (Pinus elliottii (Engelm.) var. elliottii) seed lots from the Arrowhead Seed Orchard (ASO) of the Georgia Forestry Commission (GFC) and an industrial orchard in Georgia resulted in the isolation of fungi from the affected seeds. Subsequent research in the ASO determined that the pitch canker fungus, Fusarium moniliforme Sheld. var. subglutinans Wollenw. & Reinke (FMS), and a second fungus, Lasiodiplodia theobromae (Pat.) Griff. & Maubl., were the principal pathogens associated with the seeds' deterioration. Inoculation tests confirmed that both organisms were pathogenic on maturing cones and seeds of slash pine, and that FMS could cause abortion of conelets following spray application of aqueous suspensions of the fungus to female strobili at the time of pollen

---

[1/] Research Plant Pathologist, Southeastern Forest Experiment Station, Department of Forestry, University of Florida, Gainesville, FL 32611.

[2/] Associate Professor of Forest Pathology, Department of Forestry, University of Florida, Gainesville, FL 32611.

[3/] Research Plant Physiologist, Southeastern Forest Experiment Station, Georgia Forestry Center, Route 1, Box 182A, Dry Branch, GA 31020.

[4/] Research Plant Pathologist, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Carlton Street, Athens, GA 30602.

receptivity (Miller and Bramlett 1979). In 1980 and 1981, Anderson et al. (1984) cultured slash pine seeds collected from seed orchards throughout the southern United States and found up to 30% of seeds from certain orchards to be infected with FMS. Additional studies on FMS suggested the possibility that the pitch canker disease was being introduced into nursery beds via infested or infected seeds (Barnard and Blakeslee 1980, Blakeslee et al. 1981). During this nursery research, FMS was isolated from a stored collection of pollen, suggesting that FMS-contaminated pollen may be one of the means by which this pathogen becomes established in the strobili and seeds of slash pine.

The objective of the present research was to determine whether the use of pollen artificially contaminated with conidia of FMS would result in infection of female strobili and seeds of slash pine following controlled pollination.

METHODS

In February 1982, 35 strobilus-bearing branches were tagged on a single ramet of GFC clones 17, 60, 68, 111, and 174 in the ASO. Twenty-five of these branches were bagged for controlled pollination using pollen with and without spores of FMS. The remaining 10 branches were wind-pollinated controls.

Inoculum of FMS, as conidia, was collected from cultures of two isolates of FMS (AH-2 and PM-7-5-2) that were isolated from slash pine seeds collected earlier in the ASO.

The pollen used was collected, vacuum dried, and stored in 1980. A five-clone mixture was made just prior to inoculum preparation and rehydrated overnight in a water-saturated atmosphere. The pollen was determined to be free of FMS through isolation tests prior to use.

Two different combinations of pollen and FMS conidia were prepared, one containing primarily microconidia (MIC) and the other containing mostly macroconidia (MAC). The MIC mixture was prepared by washing conidia from 8- to 10-day-old cultures of FMS, decanting excess water, mixing with talc into a slurry that was desiccated for 4 days at 2C. The mixture was then sieved, mixed with the pollen, and dispensed into plastic test tubes, 0.5 cc per tube. Inoculum concentration for the MIC mixture was approximately 5.3×10^6 conidia per cc of pollen. The MAC inoculum was prepared by growing FMS for 8 to 10 days on sterile carnation leaves placed on the surface of 1.5% water agar. The leaves were removed from the culture dishes, air dried overnight, and the conidia were scraped from the leaves into ca 2 cc of talc. The talc-conidia mixture was vigorously agitated on a mechanical vibrator, mixed with the pollen, vibrated again, and dispensed into plastic tubes at 0.5 cc per tube. Inoculum concentration for the MAC mixture was approximately 2.7×10^6 conidia per cc of pollen. The uncontaminated pollen was also dispensed into plastic tubes, 0.5 cc per tube. All pollen and pollen-spore mixtures were refrigerated immediately after preparation and transported to the ASO, where they were retained on ice in a cooler until used.

Treatments were applied to the selected, pollen-receptive strobili on February 18-19, 1982. The treatments were randomly assigned to the bagged branches on each ramet. Nine branches received each of the pollen-FMS combinations and seven branches received pollen only. Pollen combinations were applied using cyclone-type pollinators (Matthews and Bramlett 1981). Individual pollinators were used for each mixture and were kept separated. The person doing the pollinating rinsed his hands with 95% ethanol after each contaminated mixture was applied. Samples of the pollen and pollen-spore mixtures that were not used in the seed orchard were returned to the laboratory and tested for viability of both conidia and pollen 1 week after the treatments were applied.

Bags were removed 30 days after pollination. Strobili were examined for survival and symptoms of pitch canker or other damage at the time the bags were removed and at 2, 5, and 10 months after treatment. Sample strobili were collected at each observation period, except at 5 months, for cultural isolation to determine whether FMS was present. In addition, a sample strobilus from each treatment on each of the five clones was collected after 1 month for ovule dissection to determine in vivo pollen germination and indications of possible infection by FMS (Matthews and Blalock 1981).

Strobili collected for isolation were surface sterilized individually and split longitudinally. Six scales were selected per strobilus, three from each half distributed in the center portion of the strobilus. Each selected scale was cut in half midway between the apophysis and the ovules. A segment of tissue was also taken from the axis of each strobilus half. All tissues were placed on a culture medium selective for the growth of FMS. All cultures of FMS recovered from the sample strobili were tested for pathogenicity by inoculating 1- to 2-year-old seedlings of slash pine in a greenhouse and observing symptom development typical of the pitch canker disease. The strobili collected at the first observation period, 30 days after treatment, were shaken for 1 minute in 5 ml of sterile water with one drop of 10% Tween 20; the water was decanted and plated onto the FMS-selective medium.

The strobili collected for ovule observations were dissected and 10 ovules on 5 scales per strobilus were examined under a microscope. The total number of pollen grains and the number germinated were recorded for each ovule.

In January 1983, the seed orchard suffered severe damage from an ice storm. Shortly after the ice breakage occurred, 64 strobili that were still attached to broken, tagged branches were collected and subjected to the isolation procedures described above. The substantial losses from storm damage precluded additional sampling planned between 10 and 19 months.

All surviving cones were collected from the trees in mid-September 1983. Cones were placed individually in labeled, kraft paper bags, dried, and seeds were extracted. Seeds from each cone were placed on adhesive paper and radiographed to determine the condition of each seed (filled, empty, or damaged by fungi or insects).

After the seeds were radiographed, five seeds were randomly selected from each cone for isolation procedures. If a cone had five or less seeds, all were used. Selected seeds were surface sterilized for 15 minutes in 0.05% aqueous sodium hypochlorite, rinsed for 5 minutes in sterile distilled water, and cut in half longitudinally with a surface-sterilized razor blade. Seed coat segments were placed onto acidified potato dextrose agar (PDA). Gametophyte segments were surface sterilized for an additional 5 minutes in a sodium hypochlorite solution, rinsed for 2 minutes in sterile water, and placed onto the culture medium. Fungi growing from the seed parts were transferred to normal PDA for growth and identification. Isolates suspected to be FMS were transferred onto sterile carnation leaves on 1.5% water agar to confirm the identification.

RESULTS

Viability tests made on residual pollen and pollen-spore mixtures 1 week after the treatments were applied indicated a pollen germination of 86% and spore germination of both MIC and MAC sources of >95%.

Ovule Dissection--The mean number of pollen grains per ovule ranged from 1.5 for pure pollen to 2.9 for the MIC treatment, with the MAC treatment and wind pollination at 2.4 and 2.2 respectively. Pollen germination in the ovules was excellent, averaging 94% for the sources with FMS and 96% for sources with pure pollen and wind pollination. No obvious differences were observed in ovule appearance or abortion at this observation period in any of the four treatments. Macroconidia were observed in a resin droplet at the micropylar opening of one ovule from one MIC and one MAC scale of clone 17. In no ovules, however, were conidia observed in the pollen chambers.

Isolations from Sample Strobili--Cultures of FMS developed from the water used to rinse all of the MIC-treated strobili collected 1 month after treatment, but none developed from the MAC-treated, pure pollen, or wind-pollinated control samples.

Isolations from 33 sample strobili collected 1 month after pollination from the four treatments and five clones yielded cultures of FMS only from strobili receiving the MIC-pollen mixture. The tissues of the infected strobili from which FMS was isolated are shown in Table 1. All of the recovered FMS isolates were pathogenic to slash pine seedlings inoculated in the greenhouse.

Isolations from 15 strobili collected 2 months after treatment yielded a single isolate of FMS from an apophysis of a single scale from an MIC-treatment strobilus of clone 17. This isolate of FMS was not pathogenic on slash pine seedlings.

No cultures of FMS were isolated from three MIC, one MAC, and one pure pollen cones collected 10 months after treatment.

Following the ice storm in January 1983, a total of 64 strobili were collected from fallen branches that retained identification tags: 6 MIC, 14

MAC, 16 pure pollen, and 28 wind pollinated. FMS was isolated from the axis of a single MIC strobilus from clone 174. The pathogenicity test on slash pine seedlings was positive.

Table 1--Distribution of Fusarium moniliforme var. subglutinans (FMS) in tissues of slash pine strobili from five slash pine clones 1 month after MIC pollination.

GFC clone	Infected strobili	Strobilus tissue		
		Axis[1/]	Apophysis[2/]	Ovule[2/]
17	A	-	-	1
	B	-	-	1
	C	1	-	-
60	A	1	-	-
111	A	1	1	4

MIC = FMS microconidia + pollen.

[1/] Number of FMS cultures from two axis samples, one per each strobilus half.

[2/] Number of FMS cultures from three scales cut into an apophysis and an ovule half, six tissue segments per half.

The total losses of strobili from tagged branches, exclusive of those collected, from initiation of the research in February 1982 through early December 1982, are summarized in Table 2.

Unexplained losses of treated strobili were greatest in the MIC and MAC treatments on clone 17 and the MIC treatment on clone 174, where losses exceeded 20%. Within the remaining three clones, strobilus losses on the pollen only and wind controls were nearly equal or exceeded those of the pollen-spore mixtures. Losses for all treatments averaged across clones were highest for the MIC treatment (14.3%) followed by MAC (11.9%), pollen only (10.1%), and wind pollination (7.5%) (Table 2).

Seed Analysis--The average number of seeds within each of the different condition classes, by treatment, is shown in Table 3. The wind-pollinated controls produced more total seeds and more filled seeds per cone than the controlled-pollination cones, but they also had the highest average number of seeds classified as fungus damaged by radiographic analysis.

Table 2--Percentage of unexplained losses of treated strobili on five slash pine clones recorded during observations between February and December 1982.[1/]

GFC clone	Treatment			
	MIC[2/]	MAC[2/]	Pollen[2/]	Wind
17	27.6	23.1	4.8	3.3
60	6.1	3.1	12.5	0.0
68	3.7	14.8	10.0	15.2
111	13.6	14.3	16.7	11.5
174	20.7	5.0	6.3	6.9
Mean	14.3	11.19	10.1	7.5

[1/] Exclusive of strobili collected for isolation.
[2/] MIC = FMS microconidia + pollen.
MAC = FMS macroconidia + pollen.
Pollen = Pollen only.

Table 3.--Total cones and mean number of seeds per cone of different condition classes extracted from surviving cones of five slash pine clones 19 months after strobili were pollinated with pollen with and without conidia of Fusarium moniliforme var. subglutinans (FMS).

Treatment	Total cones (No.)	Seed per cone	Mean Number of Seeds[1/]			
			Filled	Empty	Damaged by fungi	Damaged by insects
MIC[2/]	51	55	45	6	3	<1
MAC[2/]	48	47	35	8	4	<1
Pollen[2/]	31	32	24	6	1	<1
Wind	59	90	70	10	10	<1

[1/] Classified by radiography.
[2/] MIC = FMS microconidia + pollen.
MAC = FMS macroconidia + pollen.
Pollen = Pollen only.

Isolation--FMS was not recovered from any of the 841 seeds that were tested for the presence of the pathogen. A total of 126 cultures were isolated from either the seed coats or the internal seed tissues. The only known pathogen recovered was L. theobromae which was present in 14 seeds (1.7%). Other fungi isolated were Fusarium sp., Pestalotia sp., Penicillium sp., and several as yet unidentified fungi.

DISCUSSION

The combined results of the viability tests made on pollen and pollen-spore mixtures 1 week after treatment, the presence and viability of pollen grains observed in the dissected ovule samples 1 month after treatment, and the number of filled seed in mature cones indicate that the pollination techniques used in this study were successful. Also, the isolation of FMS from the surface and from internal tissues of MIC-treated strobili 1 month after treatment demonstrated that infection can occur during the time of pollination. The failure to recover FMS from any of the MAC-treated strobili is difficult to interpret, but it may be related to differences in type of conidia used (microconidia vs. macroconidia) or inoculum density; conidia of FMS/cc of pollen in MIC was nearly double that of the MAC (5.3 million vs. 2.7 million spores/cc of pollen).

The greatest amount of unexplained loss of strobili during the first 10 months after treatment occurred in the MIC and MAC treatments in clone 17, and the MIC treatment in clone 174. It may be significant that clone 17 was the one from which FMS was recovered most frequently in the samples collected after 1 month, and the source of the only FMS isolate from the samples collected after 2 months. We have no explanation, however, for the failure to isolate FMS from the MAC-treated strobili of clone 17 or the MIC-treated flowers of clone 174 where, in both cases, the mortality after 10 months was considerably higher than in other treatments.

Mortality of slash pine strobili inoculated with aqueous suspensions of FMS mycelium and conidia at the time of pollen receptivity has been reported previously by Miller and Bramlett (1979), based on research in the same seed orchard. The higher infection rate obtained in this earlier research may reflect differences in environmental conditions at the time of inoculation or it may have resulted from the fact that the inoculum containing mycelium was applied in water, which could create a more favorable environment for spore germination and infection of the strobili.

In this current study, neither the radiographic analysis nor the cultural isolations of seeds extracted from the mature, treated and control cones indicated any effect of the inoculation of pollen-receptive strobili with FMS on the average number of filled or fungus-damaged seed per cone. The average number of fungus-damaged seeds per cone in the wind-pollinated controls was either approximately the same or greater than that from either of the two pollen-spore mixtures.

The overall results of this research confirm the previous research by Miller and Bramlett (1979) that, under conditions of artificial inoculation, FMS can infect female strobili of slash pine, and at least in certain

clones, may cause a significant loss of strobili. There was no indication, however, of a direct relationship between inoculation of female strobili with FMS and subsequent seed damage at maturity, at least based on the approximately 50% of the experimental cones that survived the ice storm.

Based on the results of this study and previous research (Miller and Bramlett 1979, Anderson et al. 1984), there can be little doubt that cone and seed infection by FMS does occur in slash pine and can cause significant losses, but it appears unlikely that strobilus infection at the time of pollen receptivity is a major source of seed infection. Further research is needed to determine when and how FMS and other fungi gain access to cones and seeds of slash pine.

CONCLUSIONS

1. FMS can infect strobili of slash pine when conidia are introduced at the time of pollen receptivity.
2. FMS can be isolated from asymptomatic tissues (ovules, scales, axis) of inoculated strobili.
3. Seed yields and seed infections did not appear to be significantly affected by inoculations with FMS at the time of pollen receptivity.
4. Infection of seeds by FMS apparently occurs sometime during the second season of cone growth and maturation.

LITERATURE CITED

Anderson, R. L., E. Belcher and T. Miller. 1984. Occurrence of seed fungi inside slash pine seeds produced in seed orchards in the United States. Seed Sci. & Technol. 12:795-799.

Barnard, E. L. and G. M. Blakeslee. 1980. Pitch canker of slash pine seedlings: A new disease in forest tree nurseries. Plant Dis. 64: 695-696.

Blakeslee, G. M., T. Miller, and E. L. Barnard. 1981. Pitch canker in forest tree nurseries. USDA Forest Service, SE Area State and Private Forestry, For. Bull. SA-FB/P 22, 1 p.

Matthews, F. R. and T. E. Blalock. 1981. Loblolly pine pollen grain counts by ovule dissection. In Proceedings, 16th Southern Forest Tree Improvement Conference, May 27-28, 1981, VPI & SU, Blacksburg, VA, p. 276-278.

Matthews, F. R. and D. L. Bramlett. 1981. Cyclone pollinator improves loblolly pine seed yields in controlled pollinations. South. J. Appl. For. 5:42-46.

Miller, T. and D. L. Bramlett. 1979. Damage to reproductive structures of slash pine by two seed borne pathogens: Diplodia gossypina and Fusarium moniliforme var. subglutinans. In Proceedings, Flowering and seed development in trees: a symposium, Frank Bonner, ed. USDA Forest Service, Southern Forest Experiment Station, New Orleans, LA, p. 347-355.

AN OPERATIONAL TRIAL OF SUPPLEMENTAL MASS POLLINATION IN A LOBLOLLY PINE SEED ORCHARD

T. D. Blush [1/]

Abstract.--Five ramets each of two second-generation loblolly pine seed orchard clones were supplementally mass pollinated (SMP'd) with pollen heterozygous for a rare, electrophoretically detectable marker allele. Pollen was applied once to all strobili clusters on a tree at or shortly before its period of maximum female receptivity. A pole duster was used to apply freshly processed pollen. Four ramets each of the two study clones were reserved as untreated controls to be pollinated by the orchard pollen cloud. SMP success was quantified for each ramet as the proportion of embryos fertilized by marker pollen in a 100-seed sample. Ramets of clone 11-1027 averaged 48% SMP success; those of clone 8-1048 averaged 69%. Levels of the marker allele in 100-seed samples of untreated control ramets were negligible. Differing SMP success rates among ramets of a clone are probably due to the timing of application as well as the quality of the application technique. Supplemental mass pollination appears promising as a pollen management tool in loblolly pine seed orchards.

Keywords: Pinus taeda L., supplemental mass pollination, seed orchard, electrophoresis.

INTRODUCTION

Supplemental mass pollination (SMP) is the broadcast application of desired pollen to receptive female strobili that are not isolated from the ambient pollen cloud (Bridgwater and Trew 1981). Pollen is applied with the expectation that a high proportion of ovules in treated strobili will be fertilized by applied pollen rather than by ambient pollen. Implementing SMP as a seed orchard management practice requires efficient pollen collection and processing methods, a reliable means of applying this pollen to large numbers of receptive female strobili, and, most important, a high degree of success at effecting the desired fertilizations. SMP success, the proportion of seed fathered by applied pollen, must be consistently high in operationally generated seedlots for this management technique to be economically justifiable.

[1/] Research Scientist, Genetics Group, Westvaco Forest Science Laboratory, Summerville, South Carolina.

Supplemental mass pollination is not a new idea (Wakeley et al. 1966, Franklin 1971, Woessner and Franklin 1973), but its operational application has been hampered by the lack of a suitable means to quantify success. Phenotypic markers such as hybridity (Wakeley et al. 1966) and rust resistance (Bridgwater et al. 1987) have been used with limited success. Bridgwater and Williams (1983) applied dyed pollen, followed by ovule dissection to determine the proportion of ovules containing dyed pollen grains. But this technique only indicates success at introducing pollen into the ovule. It does not quantify actual fertilization success. Perhaps the best technique for quantifying SMP success is the use of electrophoretically detectable gene markers (Hadders 1984, Wheeler and Jech 1985). Pollen is collected from clones containing a rare marker allele and used to SMP selected female clones whose genotypes facilitate electrophoretic assay of embryos. This technique provides an unequivocal means of determining paternity of SMP-derived seedlots. From this, SMP success can be reliably estimated.

This study was undertaken to estimate the level of SMP success achievable in an operational program. Fresh pollen was collected from a clone containing a rare allele, and applied to ramets of two seed orchard clones. Seed derived from this treatment was collected and assayed electrophoretically to determine levels of SMP success.

METHODS

Study Design

Study trees were selected from a second-generation coastal loblolly pine seed orchard near Charleston, South Carolina established in 1973. Nine ramets each of clones 8-1048 and 11-1027 were randomly selected and numbered from 1 to 9. Ramets 1 to 5 were SMP'd using marker pollen. Ramets 6 to 9 were reserved as open-pollinated controls.

Clones were chosen on the basis of their allozyme genotypes at the malate dehydrogenase 1 (MDH1) locus, as well as their reproductive phenologies. Clones 8-1048 and 11-1027, the female parents, are homozygous for the most common MDH1 allele (relative mobility=100) in the seed orchard population. Their diploid genotype is MDH1-100/100. The male clone is the only one of 58 seed orchard clones heterozygous for a rare fast-migrating allele, with relative mobility of 112. Its diploid genotype is MDH1-100/112. Allele MDH1-112 can be readily detected in embryos using starch gel electrophoresis. The pollen donor sheds pollen before female strobili of 8-1048 and 11-1027 are fully receptive. Female clones were receptive at the peak of seed orchard pollen flight.

Pollen Application

Freshly processed pollen was applied in spring 1985 to all strobili clusters of a tree at or shortly before the majority of female strobili were maximally receptive. Each treatment tree was SMP'd once, except for ramet 3 of clone 11-1027 which was inadvertently treated twice. Application dates are indicated in Table 1 (see RESULTS AND DISCUSSION section). All ramets of

a clone were not necessarily treated on the same day because enough processed pollen was not immediately available. Pollen was applied from a self-propelled mobile aerial lift with a Westvaco-designed pole duster, using dry nitrogen as a propellant (10 psi). The pole duster was calibrated to deliver 0.5-1.0 cc of pollen to each strobili cluster.

Electrophoretic Assay of Seed

At least thirty cones were randomly picked from throughout the crown of every SMP'd and open-pollinated tree in fall 1986. Sound seeds were extracted and cleaned, and kept separate by ramet within clone. A random sample of 100 germinated seeds per ramet was selected for assay via starch gel electrophoresis.

Electrophoresis was done using techniques adapted from Conkle et al. (1982). Germinated embryos were dissected from each seed, macerated in a phosphate buffer, and the crude homogenate absorbed onto chromatography paper wicks. Gels were 11% starch. A morpholine citrate (pH 6.1) gel and electrode buffer system was used (Conkle's "D" system). Gels were run at 60 milliamps for 4.5 hours. Gels were stained for MDH, the enzyme system resolving the gene locus containing the pollen marker allele. The diploid genotype of each assayed embryo was determined at the MDH1 gene locus. SMP success was determined as twice the proportion of embryos with diploid genotype MDH1-100/112. Since the marker pollen was heterozygous for MDH1-100 and MDH1-112, one-half of its pollen grains will carry the common allele and one-half will carry the faster-migrating rare allele. Thus it is necessary to double the frequency of MDH1-112 in the assayed progeny to estimate the number of fertilizations attributable to the marker pollen.

The basic response variable of this study is the proportion of fertilizations attributable to applied marker pollen. This proportion will be referred to as "SMP success" (%). The values are reported for each treated and control tree. This SMP success rate is derived from the 100-seed sample assayed from each study tree. The 95% confidence interval, based on the binomial distribution (Table W, Rohlf and Sokal 1969) is reported for each SMP success percentage. SMP success reported for the open-pollinated controls would, of course, be expected to be zero. However, low levels of the marker allele can be expected in the controls due to open pollination by marker pollen seed orchard ramets, or by pollination from sources located outside of the orchard that contain allele MDH1-112.

RESULTS AND DISCUSSION

SMP Success Rate

This study was designed to quantify the levels of success that can be achieved in an operational SMP program. Significant differences in SMP success rates among treated ramets are suggested by non-overlapping confidence intervals. I interpret these differences largely as a response to better application technique as we gained experience applying pollen.

The percentages of open-pollinated embryos fertilized by supplementally applied pollen is summarized in Table 1 by ramet for the two mother clones, 11-1027 and 8-1048. The presence of the marker allele in untreated control ramets is also indicated. Figure 1 presents these data for the SMP'd trees only. SMP success on ramets of 11-1027, the first mother clone to be treated, ranged from 28% to 70% with a mean of 48%. Interestingly, ramet 3, which was treated twice, assayed at 70% SMP success. But ramet 5, which was only treated once, assayed at 66% SMP success. Two applications may not significantly increase SMP success. Bridgwater and Williams (1983) concluded this in their study on loblolly pine. Wheeler and Jech (1986) also observed this in a Douglas-fir seed orchard. SMP success on ramets of 8-1048, which was treated next, ranged from 58% to 78% with a mean of 69% (Table 1 and Figure 1). SMP success rates for 8-1048 ramets are generally higher and less variable than those of 11-1027 treated ramets.

Table 1. Percentage of open-pollinated embryos fertilized by supplementally applied marker pollen on SMP'd trees and percentage apparently fertilized by ambient marker pollen on control trees. Ramet number and application date(s) are noted. 100 randomly selected embryos observed from each ramet.

Clone					
11-1027			8-1048		
SMP success (%)	ramet number	appl. date(s)	SMP success (%)	ramet number	appl. date
SMP'd					
28	1	3/27	58	1	3/29
38	2	3/27	66	2	3/29
70	3	3/28, 3/30	72	3	3/30
40	4	3/28	72	4	3/30
66	5	3/28	78	5	3/30
$\bar{X}$ = 48.4			$\bar{X}$ = 69.2		
Control					
0	6	---	6	6	---
2	7	---	2	7	---
0	8	---	2	8	---
0	9	---	4	9	---
$\bar{X}$ = 0.5			$\bar{X}$ = 3.5		

Because enough processed pollen was not always available, all ramets of a clone were not treated on the same day. However, treatment was completed within two days. Because of the study design it is difficult to ascribe differences in SMP success between treated ramets to a particular cause. However, SMP success rates for ramets 1, 2, and 4 of clone 11-1027 appear to be significantly lower than that attained in the other treated ramets (Figure 1). Figure 2 plots SMP success rate for each treated ramet as a function of the order in which that ramet was treated. The value for ramet 3 of clone 11-1027 is plotted last because this ramet was treated twice. A smooth curve through these points suggests that we were on a learning curve. By the fourth treated ramet, SMP success rate had climbed above 50% and consistently

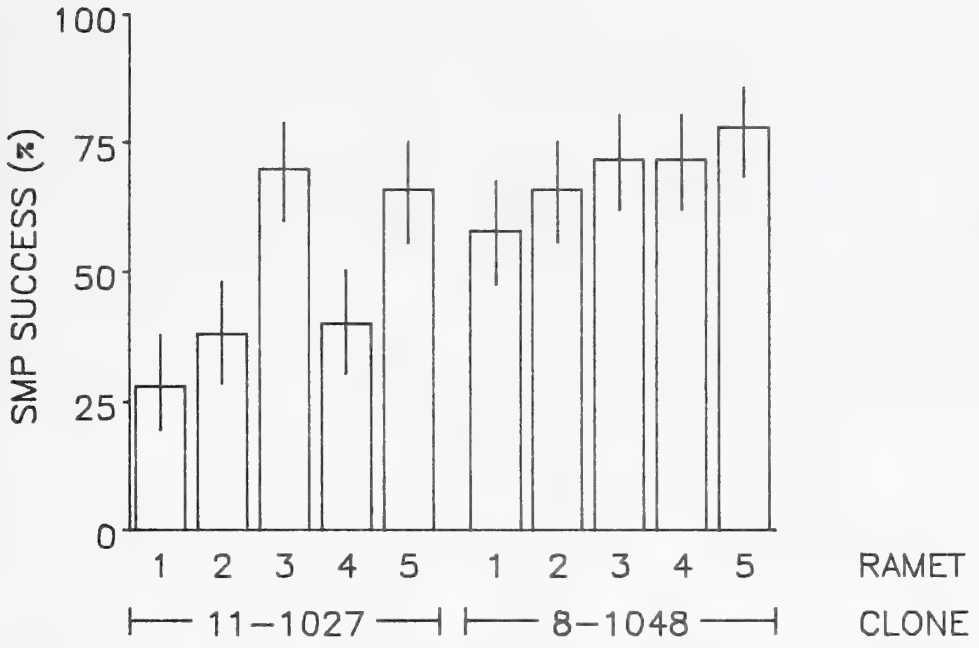


Figure 1. Levels of SMP success in treated ramets (1-5) of clones 11-1027 and 8-1048. Vertical lines represent 95% confidence interval. Ramets were SMP'd with pollen heterozygous for a rare, fast-migrating allele at MDH1 gene locus. Ramets treated once, at or shortly before maximum female receptivity. Ramet 3 of clone 11-1027 treated twice, two days apart.

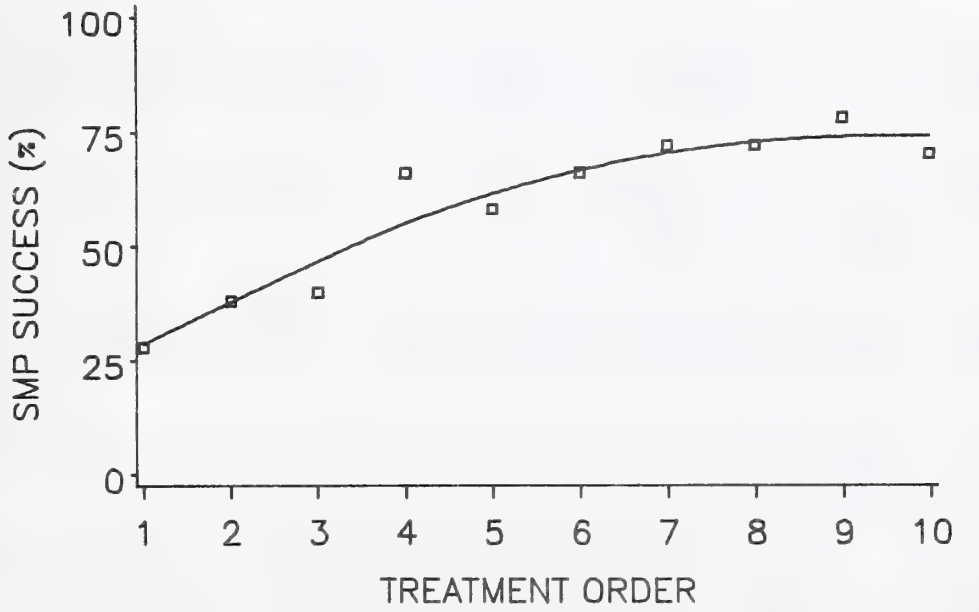


Figure 2. SMP success rates of treated ramets plotted against order of treatment. A smooth curve is drawn through data points to illustrate apparent learning curve. SMP success rate of first three ramets was below 50%. By the fourth ramet, application method had improved so that SMP success rate was consistently above 50%.

stayed above this level. This improvement with experience gained corresponds well with how the study was conducted. Initially, we applied less pollen per strobili cluster and spent less time applying pollen. Our application technique progressed, so that by the time we were treating the last study trees we applied at least 0.5 cc of pollen and held the wind shield of the pole duster over strobili clusters longer, preventing pollen from being immediately blown away. Close examination of Table 1 suggests that the timing of application may have also influenced the SMP success rate. For both clones, SMP success rates of those ramets treated the day prior to the other ramets were lower. Ramets treated last may have been more receptive, and higher SMP success resulted. Discounting the low SMP success rates of the first three trees treated, the mean SMP success rate of the last seven trees treated was 69%. This success rate may be a reasonable estimate of what can be accomplished in an operational SMP program when enough pollen is applied and sufficient time is taken to do a thorough job on each tree.

CONCLUSIONS

Supplemental mass pollination on an operational scale can be accomplished and, when using fresh pollen, success rates as high as 70% should be consistently obtainable. Results from this study and others suggest that one pollen application at or shortly before maximum female receptivity should be sufficient to achieve high fertilization success by the applied pollen. The primary determinants of success are the timing of pollen application and thorough treatment of female strobili clusters.

LITERATURE CITED

Bridgwater,F.E., D.L.Bramlett, and F.R.Matthews. 1987. Supplemental mass pollination is feasible on an operational scale. In Proc. 19th Southern For. Tree Imp. Conf. College Station, TX.

Bridgwater,F.E., and I.F.Trew. 1981. Supplemental mass pollination. P. 52-57 in Pollen management handbook, E.C.Franklin (ed.). USDA For. Serv. Agriculture Handbook 587.

Bridgwater,F.E., and C.G.Williams. 1983. Feasibility of supplemental mass pollination to increase genetic gains from seed orchards. P.78-83 in Proc. 17th Southern For. Tree Imp. Conf. Univ. Georgia, Athens, GA.

Conkle,M.T., P.D.Hodgskiss, L.B.Nunnally, and S.C.Hunter. 1982. Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For. Serv. Gen. Tech. Rpt. PSW-64, 18 p.

Franklin,E.C. 1971. Pollen management in southern seed orchards. P.218-223 in Proc. 11th Conf. on Southern For. Tree Imp. Atlanta, GA.

Hadders,G. 1984. Supplemental and controlled mass pollination in seed orchards of Scots pine (Pinus sylvestris L.). P.47-64 in Foreningen Skogstradsforadling, Institutet for skogsforbattring, Arsbok.

Rohlf,F.J., and R.R.Sokal. 1969. Statistical tables. W. H. Freeman and Co., San Francisco, CA. 253 p.

Wakeley,P.C., O.O.Wells, and T.E.Campbell. 1966. Mass production of shortleaf x slash pine hybrids by pollinating unbagged female flowers. P.78-79 in Proc. 2nd Genetics Workshop of Soc. Am. Foresters and 7th Lake States Forest Tree Improv. Conf., N. Cent. Forest Exp. Sta. Res. Paper NC-6.

Wheeler,N.C., and K.S.Jech. 1985. Estimating supplemental mass pollination (SMP) success electrophoretically. P.111-120 in Proc. 20th Can. Tree Imp. Assoc., Part 2. Quebec City, Quebec.

Woessner,R.A., and E.C.Franklin. 1973. Continued reliance on wind-pollinated southern pine seed orchards - is it reasonable? P.64-73 in Proc. 12th Southern For. Tree Imp. Conf. Baton Rouge, LA.

A SOUTHERN PINE SEED ORCHARD IN THE LOWER RIO GRANDE VALLEY OF TEXAS

C. R. McKinley[1/]

Abstract.--An experimental seed orchard of loblolly (Pinus taeda L.) and slash (Pinus elliottii Engelm. var elliottii) pine was established in the lower Rio Grande Valley near McAllen, Texas which is well outside the natural range of these species. The majority of ramets were planted in 1976 with additional material established in 1977. Flower counts made from 1979 through 1982 indicate that a higher number of loblolly flowers per ramet were produced in the South Texas Orchard than in an operational orchard established at the same time in southern Arkansas. Seed analyses did not show the presence of insect pests common to orchards located in the species' natural range.

Additional keywords: seed production, flower production, orchard management.

Forestry industry, public agencies, and universities are heavily involved in efforts to improve commercial tree species through selection and breeding. Progress is being made and production seed orchards are common throughout the United States.

Smith (1975) reported some South African seed orchards have achieved seed yields for southern pines which are several-fold above those obtained within the southern pine region of the United States. Earlier flowering in South Africa may offer a time advantage of up to 30 percent. These advantages are economically significant and would appear to justify use of this concept to maximize return on tree improvement investments. However, political, social, economic and logistical considerations lessen the attraction of the South African programs.

This paper summarizes efforts to establish a southern pine seed orchard in a sub-tropical environment. A limited review of this effort was earlier published by Richmond and McKinley (1986).

ORCHARD ESTABLISHMENT

Serious review of the technical aspects of a proposal to duplicate the South African program was begun in 1976 by a group of cooperating organiza-

---

1/ Associate Geneticist, Texas Forest Service and Assistant Professor, Forest Science Department, Texas A&M University, College Station, Texas.

tions[2/]. Several prospective areas in the United States were considered before a site was selected in the lower Rio Grande Valley of Texas. Primary criterion was a sub-tropical climate outside the natural range of loblolly and slash pine. The area is located near McAllen, Texas and is approximately 400 km from native southern pines.

The orchard was designed with a wide geographic representation of genetic material. Five loblolly blocks and one slash block were established with each block containing clones from a single region (Table 1). Clones were selected to provide a range of poor to good female flowering behavior. Scion material was provided by members of the North Carolina State University Tree Improvement Cooperative, the Florida Tree Improvement Cooperative and the Western Gulf Forest Tree Improvement Program.

Table 1.--Genetic composition of the South Texas seed orchard at time of establishment (1976-1977).

Species	Geographic Origin	Number of Ramets
Loblolly		
	Southeast Texas	45*
	North and South Carolina	36
	Maryland-Virginia	45*
	Mississippi-Louisiana	43*
	Arkansas	43
Slash		
	Texas-Louisiana-Florida	45
	Total	257

* One non-grafted seedling included in this total.

The orchard was first planted in June 1976 with a total of 140 trees. A spacing of 9.5 m x 9.5 m was used. Although summer planting is not recommended, the orchard was established at this time in order to avoid delay. To provide for perhaps earlier and better pollination, "pollinator clones", known to be high volume pollen producers, from the appropriate region and species were interplanted in 1977. A total of 117 of these additional grafts were used.

ORCHARD MANAGEMENT

While a considerable number of problems were expected as in conventional southern pine orchard management, several unexpected difficulties were encountered in the experimental area.

[2/] Cooperating organizations included: Arkansas Kraft Corp.,Champion International Corp., International Paper Co., Kirby Forest Industries, Inc., Olinkraft Corp. (now Manville Forest Products Corp.), Owens-Illinois, Inc., Nekoosa Papers, Inc., Potlatch Corp., Boise Southern Co., Temple-Eastex Forests, Texas Forest Service, and Weyerhaeuser Co.

The strong prevailing east to southeast wind was observed to have an adverse effect on the young trees. Many trees began to lean with the windward faces of developing crowns abnormally sparse and open. Temporary relief was gained by erecting burlap shields on the windward sides of young trees. These shields required considerable maintenance and appeared to become less effective as the trees grew.

The original irrigation system consisted of impact type sprinklers on five-foot risers. However, a water supply high in mineral content combined with the need for frequent watering (rainfall averages 56 cm/year) resulted in a calcium and sodium deposit on tree foliage. This problem was alleviated by replacing impact sprinklers with individual ground level bubble emitters at each ramet.

Other treatments included the application of elemental sulphur to all ramets to lower soil pH and the soil application of mycorrhizae to several ramets demonstrating poor vigor. Nitrogen fertilizer as ammonium nitrate was applied to part of the orchard in 1981 and 1982 in an effort to stimulate flower production. Fertilization also included the application of triple-superphosphate to improve tree growth.

FLOWER PRODUCTION

Female flowers (strobili) were observed in 1978. Counts of female flowers were made from 1979 through 1985 (Table 2), although the 1982 through 1985 counts were estimates as the trees were so large that it was difficult to see all flowers. Flower production varied by geographic source with the Louisiana-Mississippi and Arkansas loblolly producing the most flowers. As is commonly observed in the natural range of the two species, slash pine produced fewer flowers at these early ages than loblolly. Slash data was, therefore, not further analyzed.

Early production of female flowers was obtained in the South Texas Orchard. However, the data do not readily indicate if this production is more abundant than that expected in orchards established within the natural pine range. To compare loblolly flower production, data were obtained from the Potlatch Corporation orchard at Warren, Arkansas. This orchard is located within the natural range of loblolly pine. It was also established in 1976 and represents a geographical contrast to the South Texas location. While identical treatments were not applied to the two orchards being considered, both orchards were managed to stimulate early growth and flower production.

Flower counts for the Potlatch orchard were from a 20 percent sample of the total 16 ha. for the years 1979-1982. Flower counts for the South Texas orchard were from the 100 percent tally. Results indicate that higher loblolly female flower production was obtained in the South Texas orchard (Table 3). Specifically, 1982 data demonstrate that the South Texas orchard produced about 15 times more flowers per ramet that the Potlatch orchard and produced 22 times as many flowers per ramet when the Arkansas block is compared to the Potlatch orchard. These results are similar to those of Schmidtling (1978) who reported that an increase in cone production of loblolly orchards could be expected in the more southerly latitudes. It is believed that a significant reason for more flowering in South Texas is the presence of

Table 2.--Flowering summary for the South Texas seed orchard for years 1979-1985.

	1979	1980	1981	1982	1983	1984	1985
Loblolly							
Southeast Texas							
No. Ramets	35	30	25	25	24	25	25
Mean Flowers/Ramet	3.7	10.2	11.5	42.7	38.3	198	241
North and South Carolina							
No. Ramets	20	17	16	14	13	15	14
Mean Flowers/Ramet	1.4	5.8	4.6	36.6	38.5	168	273
Maryland-Virginia							
No. Ramets	38	31	31	29	29	31	31
Mean Flowers/Ramet	2.4	6.5	8.8	29.1	42.1	116	141
Mississippi-Louisiana							
No. Ramets	34	32	32	32	30	32	32
Mean Flowers/Ramet	9.4	23.8	21.3	69.2	64	287	313
Arkansas							
No. Ramets	37	36	34	32	32	35	35
Mean Flowers/Ramet	6.8	23.1	31.5	66.5	69.2	371	419
Slash							
Texas-Louisiana-Florida							
No. Ramets	33	31	30	27	27	29	29
Mean Flowers/Ramet	1.0	2.7	2.1	19.5	32.1	83	123

Table 3.--Flowering comparison for loblolly pine in the South Texas seed orchard and Potlatch Corporation seed orchard for years 1979-1982.

	1979	1980	1981	1982
South Texas seed orchard				
No. Ramets	164	146	138	132
% Ramets with Flowers	63.4	77.4	83.3	97.7
Mean Flowers/Ramet	5.0	15.0	17.3	51.2
Potlatch Corp. seed orchard				
No. Ramets (20% sample)	464	464	464	464
% Ramets with Flowers	23.7	9.5	33.0	29.3
Mean Flowers/Ramet	1.0	.3	2.1	3.1

multiple whorls of flowers in the same flowering season. This phenomenon has not been reported in more northern orchards and was fairly common in South Texas.

SEED PRODUCTION

To evaluate potential seed production, a pollination study was begun in 1979. The purposes were to determine if foreign pollen was reaching the orchard in sufficient quantity to result in pollination and to determine if seed or cone insects were present in the orchard.

Results of this study for 1979, 1980, and 1984 are presented in Table 4. Although both slash and loblolly were treated only the loblolly data are presented due to a relatively low survival of slash cones. While these data are not definitive, they do suggest that little, if any, pollen reached the orchard from outside sources.

Table 4.--Summary of South Texas seed orchard loblolly pollination study for 1979, 1980 and 1984.

Year	Treatment	No. Cones	Mean Seeds/Cone	Mean Filled Seeds/Cone
1979	Tagged Only	0	-	-
	Bagged Only	0	-	-
	Mass Pollinated	37	33.6	21.5
1980	Tagged Only	7	0	0
	Bagged Only	0	-	-
	Mass Pollinated	41	52.6	42.5
	Bagged/Pollinated	50	10.7	8.1
1984	Tagged Only	398	21.8	15.8
	Mass Pollinated	661	26.2	18.5

Cones were collected each year with seed extracted for radiographic analyses. Examination indicated that no seed or cone insects were present in the orchard.

SUMMARY

The experimental orchard was begun by a group of cooperating organizations in 1976. While several unexpected problems arose in management of the orchard, it is believed that the establishment of an operational orchard in this or similar location is feasible.

Loblolly flower counts made from 1978 to 1985 indicate that the experimental orchard produced considerably more flowers than a comparable orchard located in southern Arkansas. However, a detailed comparison is not possible due to different genetic compositions in the two orchards. Genetic material from Louisiana-Mississippi and Arkansas regions produced the greatest number of female flowers per ramet in the experimental orchard. A pollination study

suggested that a minimal amount of outside pollen was present. Radiographic analysis of seeds did not show any seed or cone insects.

These results suggest that pine seed orchards in South Texas or other subtropical climate may produce sufficient flowers to encourage further development, particularly when the lack of natural seed predators and contaminating foreign pollen are considered.

LITERATURE CITED

Richmond, G. R. and C. R. McKinley. 1986. An Experimental Seed Orchard in South Texas. J. of Forestry 84(7):19 and 43.

Schmidtling, R. C. 1978. Southern Loblolly Pine Seed Orchards Produce More Cones and Seeds Than Do Northern Orchards. In: Proc. Flowering and Seed Development in Trees: A Symposium. May 15-18, Mississippi State University, Starkville, MS. p. 177-186.

Smith, H. D. 1975. Potential for Foreign Production of Pine Seed for the United States. Proc. 13th Southern Forest Tree Improvement Conf., June 10-11, Raleigh, NC. p. 27-36.

EFFECT OF A CLONAL ROW ORCHARD DESIGN ON THE SEED YIELDS OF LOBLOLLY PINE

D. L. Bramlett[1/] and F. E. Bridgwater[2/]

Abstract.--When ramets of the same clone are planted in a clonal row, the frequency of self-pollination is expected to increase. With an increase in self-pollination, the filled seed yield would be expected to decrease as a result of increasing the frequency of homozygous embryonic lethal alleles. Pine species are buffered against the deleterious effects of self-fertilization by the large number of embryonic alleles in the population with each individual tree having a different set of alleles. When an individual ovule contains both self and outcross pollen, a viable seed can be produced if the self-fertilized embryo aborts but the outcross-fertilized embryo survives. Thus, in a clonal row orchard, self-pollination increases but the impact on seed yields is expected to be relatively low. Experimental data indicated no significant reduction in the percentage of filled seeds from clonal rows when compared to a random design.

Additional keywords: Pinus taeda, pollen, self-pollination, embryonic lethal alleles.

INTRODUCTION

The amount of pollen reaching a ramet in a seed orchard is a mixture of (1) self pollen from the same ramet, (2) self pollen from other ramets of the same clone, (3) outcross pollen from neighboring ramets, (4) outcross pollen from non-neighboring ramets, and (5) background pollen from outside the orchard. It is important to determine the relative quantities of pollen from each of these sources to evaluate the impact of inbreeding in a seed orchard.

Unfortunately, quantifying each pollen component is difficult. Major problems are: (1) determining the phenology of each clone in the orchard and (2) determining the pollen dispersal patterns which vary as a function of relative humidity, temperature, wind direction, and wind velocity. Because

---

[1/] Research Plant Physiologist, Southeastern Forest Experiment Station, Macon, Georgia,

[2/] Research Geneticist, Southeastern Forest Experiment Station, Raleigh, North Carolina.

The authors thank Weyerhaeuser Company for the support of this research.

female strobili (flowers) may be receptive to pollen for a week or more, some of these factors may have compensating effects. For a given ramet, the timing of female flower receptivity relative to the phenology of nearest neighbors, with other orchard clones, and with background pollen is the single most important factor.

Another factor in the success of embryo and seed development from the various sources of pollen is the action of recessive embryonic lethal alleles. The presence of embryonic lethal alleles in each individual mitigates against self progenies by effectively eliminating large numbers of self-fertilized embryos. With 10-12 embryonic lethal alleles, the probability is high (about 90%) that self-fertilized embryos will have at least one homozygous lethal and that the embryo will abort. With multiple pollen grains per ovule and multiple archegonia available for fertilization, self-fertilized embryos can abort and yet a filled seed develop from an outcross fertilization within the same ovule. Thus, mixtures of self and outcross pollen produce more successful outcross-pollinated embryos than self-pollinated embryos. The number of pollen grains per ovule, the ratio of self to outcross pollen, the numbers of archegonia per ovule, the number of embryonic lethals of the parents, all affect seed yields and genetic quality.

The objective of this study was to compare a random seed orchard design with clonal row designs. We compared self-pollination rates for a seed orchard with ramets of clones randomly assigned to positions to different clonal row designs. Self-pollination rates were estimated from a model using hypothetical values. Empirical data from one of the clonal row designs and the random design were used to test the model.

SEED ORCHARD MODEL DEVELOPMENT

We compared the relative amounts of pollen from the six different sources for four different seed orchard designs (table 1). The seed orchard designs include: random design with ramets planted on a 30 x 15 foot spacing; clonal row (CR30X15) with clones planted 30 feet apart and ramets within rows 15 feet apart; clonal row (CR15X30) with clones planted 15 feet apart and ramets within rows 30 feet apart; and clonal row (CR30X30) with both clones and ramets within rows planted 30 feet apart. These hypothetical values were developed as follows:

Self-pollination by the same ramet was assumed to be 15% for all of the designs studied except CR30X30. An arbitrary value of 17.5% was used for self-pollination by the same ramet at the least dense spacing since the proportionate contribution of pollen from other sources would logically be diminished. The proportion of pollination by unrelated, non-orchard pollen was assumed to be 40%. The proportions of pollinations from all other sources within the seed orchard were assumed to be proportionate to the frequencies of ramets of each type of male parent.

Frequencies were estimated for pollinators equal to or less than 90 feet from a target female and greater than 90 feet from a target female. This permitted weighting pollinations nearer the target tree more heavily than those farther than 90 feet from the target female.

The nearest neighbors are more important contributors to the pollen supply (Koski 1971). This author reported that 60% of pollen parents were within 32 m of a target female tree. This figure included self-pollinations by the target female. We used this percentage to estimate weights for frequencies of pollen parents within 90 feet and farther than 90 feet from the target female.

Thus, in the random design there were no ramets of the target female closer than 90 feet; but the frequency of ramets of that clone farther away was (100-1)/(4000-1) = .025. These ramet frequencies were multiplied by the weights estimated from Koski's relationship to arrive at the values in table 1.

Table 1. Hypothetical pollen distribution models for 10-year-old loblolly seed orchards with random vs 3 clonal row designs with 3 spacing configurations.[1/]

SOURCE OF POLLEN	ESTIMATED PERCENTAGE OF POLLEN FOR TARGET RAMET			
	RANDOM[2/]	CLONAL ROW DESIGNS[3/4/]		
	DESIGN	CR30X15	CR15X30	CR30X30
	(Percent)			
Self Same Ramet	15.0	15.0	15.0	17.5
Self Other Ramets				
Within 90 Ft	0.0	5.3	2.6	4.7
Outside 90 Ft	0.5	0.4	0.5	0.5
Outcrosses				
Within 90 Ft	23.8	18.5	21.2	17.2
Outside 90 Ft	20.6	20.8	20.7	20.1
Background	40.0	40.0	40.0	40.0
Total	100.0	100.0	100.0	100.0
Total Self	15.5	20.7	18.2	22.7

[1/] Orchard contains 40 clones with 100 ramets of each clone.

[2/] Ramets of the same clone are not closer than 90 feet.

[3/] Clonal rows are 20 trees long with 5 rows for each clone. A block consists of 1 row per clone for each of 40 clones.

[4/] First number = distance between clonal rows; second number = distance between ramets of the same clone within rows.

With clonal row designs, the number of ramets of the same clone within 90 feet of a target female ramet increased (table 2). Thus, higher frequencies are expected for self-pollination with clonal row designs. Estimates ranged from 15.5% self pollination with random designs to 22.7% with a clonal row of twenty ramets spaced 30 feet x 30 feet (CR30X30) table 1.

Table 2.--Number of self and outcross ramets within a circle with a 90-foot radius comparing random design to three clonal row designs of different spacing configurations.

PLANTING DESIGN	MAXIMUM NUMBERS OF RAMETS WITHIN A 90 FOOT RADIUS		
	TOTAL	SELF	OUTCROSS
Random 15x30	55	1	54
Clonal Row CR30X15	55	13[1/]	42
Clonal Row CR15X30	55	7	48
Clonal Row CR30X30	29	7	22

[1/] The number of self for clonal row designs are maximum values because ramets on the ends of clonal rows have fewer ramets within 90 feet.

Based on these estimates of the pollen source components, the percentage of self pollen increased with clonal row designs but not by as much as one might think. We tested this model using empirical data from a random design seed orchard compared to one of the clonal row designs.

TESTING THE MODEL

Seed yields and the percentages of filled seeds per cone were observed in the Weyerhaeuser Company's NC Loblolly Seed Orchard at Lyons, Georgia. A portion of the orchard consisted of 18 clonal rows, each with 20 ramets per clone. The clonal rows were 15 feet apart and ramets within rows were 30 feet apart (CR15X30). The trees were grafted in 1977 and planted in 1978. The study trees were approximately 26 feet tall and 8 inches d.b.h. in 1985.

With 30 feet between the ramets in a clonal row, trees in row positions 1-4 and 17-20 had fewer ramets of the same clone within 90 feet than ramets in row positions 5-16. Five ramets from five clones were selected as observation trees from row positions 1, 2, 3, 4 and 10 to evaluate the effect of row positions. These ramets had respectively 3, 4, 5, 6, and 6 ramets of the same clone within a 90-foot radius. Fiberglass screen cages were installed around three clusters of developing cones on each study ramet in May 1985 to prevent

damage from insects (Bramlett et al. 1976). Mature cones were collected in October 1985 and air-dryed until open. Seeds were extracted by hand. The axes of cones that opened poorly were drilled and their scales separated. More than 95% of the seeds were extracted using this method. All extracted seeds were radiographed and scored as filled or empty. A total of 157 cones from the caged branches were analyzed from the 25 sample trees. In addition, 134 uncaged cones from the same clonal row ramets were collected, and their seeds extracted and analyzed in the same manner. Seeds were analyzed from 78 cones from 13 ramets in a RD orchard.

RESULTS

Clonal row vs random design

Neither caged nor uncaged cones from the clonal row ramets had significantly different numbers of extracted seeds, from uncaged cones collected from RD ramets (table 3). Mean values were 100, 107, and 99 respectively for caged clonal-row ramets, uncaged clonal row-ramets, and uncaged random design ramets. These data indicate that the reproductive capacity for the total number of seeds was not related to caging of developing cones or to orchard planting design. Clonal effects, as expected, strongly influenced the number of extracted seeds per cone. Clone A and B produced significantly more seeds per cone than clones C, D, and E (table 3).

Filled seeds per cone were increased by caging the cones from the time of fertilization to cone maturity (Table 3). The mean value of 79 filled seeds per cone for caged, clonal row trees was significantly higher than the filled seed yield per uncaged cone from the same ramets in the clonal row (49) or from different ramets of the same clones in the RD part of the same orchard (53). The difference is attributed to insect attack on the uncaged cones during the summer of cone development. Seedbug attack during this time period produces empty seeds while ovules damaged prior to fertilization appear as aborted ovules rather than empty seeds. (Bramlett et al. 1977)

Empty seeds from caged cones are caused by factors other than insects. The action of homozygous embryonic lethal alleles is considered the primary cause when insect damage is eliminated. Other possible causes of empty seeds are fungal damage, poor pollen vigor, and abnormal development.

The percentage of filled seeds per cone was used to estimate the impact of self pollination on seed yields. Caged clonal-row cones had significantly higher percentages of filled seeds (79) than noncaged clonal row cones (50) and random design cones (52). Assuming that both were reduced the same by insect damage, there was no significant reduction in percentages of filled seeds from clonal rows.

Table 3.--Extracted Seed, filled seed, and percentage of filled seed from clonal row ramets and random-design ramets of the same clones.

CLONE	CLONAL ROW CAGED	CLONAL ROW UNCAGED	RANDOM DESIGN UNCAGED	CLONAL MEAN
		EXTRACTED SEEDS PER CONE		
A	124	134	127	129 a
B	109	127	121	119 a
C	83	83	76	81 b
D	99	99	67	92 b
E	84	86	101	89 b
MEAN	100 a	107 a	99 a	102
		FILLED SEEDS PER CONE		
A	100	58	54	75
B	87	60	73	73 ab
C	59	30	34	42 c
D	85	64	42	70 ab
E	63	39	59	54 bc
MEAN	79 a	49 b	53 b	62
		PERCENTAGE FILLED SEEDS		
A	81	43	41	58 b
B	78	46	59	62 b
C	72	36	46	52 b
D	86	65	57	74 a
E	77	42	53	59 b
MEAN	79 a	50 b	52 b	61

The expected differences in percentages of self-pollinations between a random design and a clonal row design with 15 feet between rows and 30 feet between ramets was 18.2-15.5 = 2.7% (from table 1). The expected reduction in the percentage of filled seeds assuming an average of 10 embryonic lethal alleles is 1.5% (Koski, 1982). The non significant difference measured in the empirical study (51.6-49.9 = 1.7%), was surprisingly close to the expected value.

The effect of row position on seed yields

There was no significant difference in the percentage of filled seeds among the 5 sample ramets in different positions in rows (table 4). Mean values varied from 82.8 percentage filled seeds in row position 2 to 76.6 for row positions 4 and 10. Individual clones had significantly different mean values ranging from 71.4 percentage filled seeds for Clone C compared to 86.1 percent filled for Clone D.

Table 4.--Percentage of filled seeds from five row positions in a 20-tree loblolly pine clonal row orchard

CLONE	NO. OBS	ROW POSITION 1	2	3	4	10	AVG
		Percent					
A	21	81.5	68.7	77.3	92.1	81.3	80.2 ab
B	36	66.0	87.9	73.6	77.6	91.3	74.5 b
C	36	85.5	78.7	69.7	69.6	60.3	71.4 c
D	33	89.7	90.0	86.0	80.9	83.9	86.1 a
E	31	76.4	81.3	82.8	69.6	72.9	76.7 bc
	157	78.8 a	82.8 a	78.1 a	76.6 a	76.6 a	78.6

CONCLUSIONS

Results from this study indicated no significant differences in seed yields or percentages of filled seeds between ramets in clonal rows and ramets of the same clone in a random orchard design. Furthermore, within the clonal rows, position had no significant effect on the percentages of filled seeds per cone.

A theoretical pollen distribution model to predict the percentage of filled seeds from different seed orchard designs was developed and found to accurately predict the observed decrease in the percentage of filled seed between two different orchard designs

Thus, the concept of clonal row seed orchard designs should not be rejected based on presumed large increases in self-pollination and self-fertilization.

LITERATURE CITED

Bramlett, D.L., E.W. Belcher, Jr., G.L. DeBarr, and others. 1977. Cone analysis of southern pines: a guidebook. Gen. Tech. Rpt. SE-13 Asheville NC: USDA, Forest Service, Southeast. For. Expt. Sta.

Bramlett, D.L. and F.E. Bridgwater. 1986. Segregation of recessive embryonic lethal alleles in a F_1 population of Virginia pine. pp 401-409. In: Proc. of IUFRO Conf. on Breeding Theory, Progeny Testing, and Seed Orchards; Oct 13-17, 1986; Williamsburg, VA. N.C. State University, Raleigh, NC.

Bramlett, D.L., W.G. Lewis, and G.L. DeBarr. 1976. Screen cages to protect control-pollinated pine cones from seedbugs. Tree Planter's Notes 28(2):25-28.

Koski, V. 1970. A study of pollen dispersal as a mechanism of gene flow in conifers. Comm. Inst. For. Tenn. 704: 78 pp.

Koski, V. 1982. How to study the rate of inbreeding in populations of Pinus sylvestris and Picea abies. Silva Fennica 16: 83-87.

DESIGN, ESTABLISHMENT, AND MANAGEMENT OF A BLACK ALDER (*ALNUS GLUTINOSA* L. GAERTN.) SEED ORCHARD

R. B. Hall and R. N. Nyong'o [1/]

Abstract.--Improved selections are being developed for use in the intensive culture of biomass for energy, nitrogen-fixing nurse crop plantings, and for soil reclamation plantings. As a complement to this selection work, we have been studying seed-orchard alternatives for the species by establishing and managing an experimental orchard. Five-year-old selected trees were moved by tree spade to establish the orchard. It has been expanded through the use of rooted cuttings. Our initial design of this orchard takes into account the existence of a self-incompatibility system in *Alnus* and tries to balance current uncertainties as to whether inter- or intra-provenance crosses should be favored. This design will be modified in place as our knowledge improves. Seed collections made from seven typical seed orchard trees in the fall of 1985 and 1986 gave a total seed yield of 3.6-7.4 million seeds (5630-11,693 g) with germination rates of 23-74%. Because of delayed fertilization in alder, it is likely to be important to be able to irrigate an orchard during early summer droughts to protect seed viability. Pollen dispersion from our plantings indicate that an isolation strip 710 m wide is needed to exclude 91% of the pollen from nonselected trees. Early fall is the best time for seed collection. A systemic insecticide has been used to control a defoliating insect in the orchard. We have begun studies to determine the feasibility of periodic coppicing of the seed orchard trees to facilitate seed collection and maintain smaller trees.

Additional keywords: Breeding, flowering, coppice growth.

Currently, most European alder planting stock is produced for use in mine-spoil reclamation. In 1985, 484,000 *A. glutinosa* seedlings were shipped from state nurseries in the northeastern United States, ranking alder as eighth in hardwood popularity behind such species as walnut, red oak, yellow poplar, and the ashes (Scholtes 1985).

Several projects conducted under the U.S. Department of Energy's Short Rotation Woody Crops Program (SRWCP) have identified *Alnus* as a promising genus for the intensive culture of biomass because of its rapid growth and

---

[1/] Professor and Research Assistant respectively, Department of Forestry, Iowa State University, Ames, Iowa 50011. Journal Paper No. J-12725 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50011. Research performed under Station Project 2210 (a contributing project to North Central Regional Project NC-99) and Subcontract No. 19X - 43391C with Oak Ridge National Laboratory under Martin Marietta Energy Systems, Inc., Contract DE-AC05-840R21400 with the U. S. Department of Energy.

symbiotic nitrogen-fixation capabilities (Ranney et al. 1985). Black alder is being considered for biomass energy production in the Midwest, Lake States, and South/Southeastern Regions where it is classified in the SRWCP's "group 2" of species, with maximum measured yields of 4 to 10 Mg/ha/yr of dry-weight biomass. In addition, alders can fix 150+ kg/ha/yr of nitrogen and this has created interest in finding selections compatible for use in mixture with Populus or other SRWC species. Black alder is also better than most species in its ability to coppice. The genus Alnus is very diverse with considerable alternatives and potential for improvement (Hall and Maynard 1979, Hall et al. 1983). However, no improved alders are currently available. The State of Kentucky did attempt to establish an alder seed orchard, but it failed because no selection for Phomopsis resistance had been practiced (Oak and Dorset 1983).

We began genetic improvement work with Alnus in 1976. We organized a range-wide collection of germplasm for Alnus glutinosa and have grown selected populations of a few other Alnus species. In 1979 and 1980, we established provenance tests of these materials in northern Wisconsin, central Iowa, and southern Illinois, and through cooperators, we provided material for 25 additional provenance tests in many of the eastern United States, Oregon and Washington, and Canada (Robinson and Hall 1981). We have published several articles summarizing our test results and developing a strategy for genetic improvement in the genus (Hall and Maynard 1979, Maynard and Hall 1980, Robison and Hall 1981, Hall et al. 1983, Hall and Miller 1983). We have begun the selection and breeding work with these populations. In this paper, we report our observations on seed orchard design, establishment, and management.

METHODS

Four separate plantings of black alder were used. Observations on age-to-flowering and early seed production for seedlings and coppice growth were made in a range-wide provenance test planted in 1979 and described elsewhere (Hall et al. 1983). Seventeen selected 5-year-old trees from this provenance test were lifted with a large Vermeer tree spade and were replanted as a seed orchard at a 5x5 m spacing in July 1984. This seed orchard was established at a distance of 635 m (2080 ft) from our other alder plantings at the University's Rhodes Farm in central Iowa. In the spring of 1986, the seed orchard was enlarged to a total size of 46 trees by using rooted cuttings of our selections. The additions were put in using the permutated neighborhood design to promote panmixia (Faulkner 1975). In 1981, we also established a small clonal plantation at our Rhodes Farm by using 10 replications each of 26 clones planted as single tree (rooted cuttings) plots on a 1.5x1.5 m spacing (Hall et al. 1983). All these Rhodes Farm plantings are on a gently undulating bottomland area with predominantly a Colo silty clay loam soil type.

Because our experimental seed orchard had not yet come into full production, we used a set of black alder trees at the Iowa State Nursery in Ames to estimate potential seed-orchard production and thereby calculate the acreage of seed orchards needed to support future planting needs. The trees at the state nursery were transplanted in 1976 and coppiced in 1978 or 1980 in a fashion and at a spacing very similar to the way our seed orchard has been

treated. The trees recovered from the transplant shock, coppiced well, are flowering heavily, and were at a size we consider optimum for seed colletcion (ca. 8 m tall). The total seed crop from each of seven trees was harvested in the fall of 1985 and again in 1986.

To determine maturation rates in black alder strobili, we made weekly collections from different trees in each of three clones in our clonal plantation. In 1985, these collections were made from Sept. 4 to Oct. 9. In 1986, a longer period, from July 31 to Oct. 15, was sampled. Harvested strobili were air-dried for one week in a greenhouse bay, and seed were extracted by hand-dissection or by shaking opened strobili in a paper bag. Germination tests were run on four replications of 100 seeds for all seedlots at the Iowa State Seed Testing Lab using a 30^{0} C day and 20^{0} C night schedule in lighted, high-humidity (>90%) chambers. To study pollen dispersal, three transects of pollen traps were established at our provenance, clonal, and seed orchard sites in March 1987. The pollen trap method of Hoekstra (1965) was used, and 10 systematically located fields of view, 0.049 mm^2 each in area, were studied under 400X magnification to determine an average pollen count.

RESULTS AND DISCUSSION

Design

Our recent experience confirms our earlier suggestion (Robison and Hall 1981) that either seedling or clonal seed orchards can be used for black alder. In our provenance test, most of the best phenotypes began flowering in their third growing season. We collected an estimated 390,000 seeds from 83 trees at the end of their fourth growing season. When trees are coppiced, they return to seed production at the end of their second or third growing season, and, as shown later, they are capable of producing commercial quantities of seed by at least 6 years of age. Likewise, trees propagated by rooting cuttings begin flowering in their third year in the field, and from that time on, they flower at least as abundantly as seed-origin trees do. We also have done grafting of flowering scions to greenhouse stock plants since 1983 with varying success. Most of these plants have been kept under greenhouse conditions for only 1 year. Approximately 75 grafted trees have been carried through winter conditions in shade frames or transplanted to the field. Graft survival has been poor; of those surviving, less than 10% have flowered in their second year and maintenance of scion/understock identity is a continual problem. Therefore, it seems that the use of rooted cuttings is the better approach to cloning this species for seed orchard purposes.

The best advantages of both seedling and clonal seed orchards can be combined into one operation. Seedlings can be grown in provenance or progeny tests to their proper age for evaluation. Selected trees can then be lifted and moved by tree spade to a seed orchard. Thus, a well-designed, properly spaced, but small, seedling seed orchard can be created. When we did this with our provenance test selections, the trees did produce seed the year that they were moved and the year following. This offers the opportunity to begin producing controlled- or open-pollinated seed right away for establishing advanced generations and progeny tests of the orchard's potential. At the same time, the selected trees can be propagated by cuttings. When those

ramets are ready to go to the field, they can be used to expand the operation into a clonal seed orchard that should produce sufficient quantities of seed for commercial use within 3 to 6 years.

Alders are self-incompatible, evidently with an S-allele system (Hagman 1975), so selfing is not a concern in the design of seed orchards. However, the level of incompatability between relatives is not well known and our breeding studies have not progressed to the stage at which we know whether to emphasize crossing between geographic populations or to select and breed within them. In our experimental seed orchard, we initially established small groupings of trees selected from each provenance. When the orchard was expanded with rooted ramets, we emphasized crossing between individuals of different provenances. When progeny test results are available to indicate the preferred crossing pattern, we can reorganize our existing orchard by moving trees with a tree spade or by starting new rooted cuttings.

Seed production in young alder trees can be very abundant (table 1). The seven trees sampled produced almost 1.5 million viable seeds in 1985 and more than 3.4 million in 1986. Amounts of seed produced and germination rates of the seed do vary by individual tree and by year. However, all trees in this study, and others that we have observed, produce sufficient seed that fecundity need not be a consideration in choosing trees for seed orchards. We anticipate harvesting the seed from stems that are 5 to 10 years of age. Hence, the seed collections reported in table 1 for trees at ages 6 to 9 should be fairly typical. With orchard trees spaced at 5x5 m (16.4 ft) and an average annual production of 1.2+ kg (2.7 lb) of seeds/tree, we should be able to produce 496 kg/ha (432 lb/A) of seed.

Table 1.--Measurements of potential seed orchard production based on trees at the Iowa State Tree Nursery.

Tree No.	1985 Stem Age	1985 Ht. (m)	1985 DBH (cm)	Strobili Produced: 1985 Bushels	Strobili Produced: Air Dry Weight (g) 1985	Strobili Produced: Air Dry Weight (g) 1986	Seed Produced: Fresh Weight (g) 1985	Seed Produced: Fresh Weight (g) 1986	Seed Produced: Germination Percent 1985	Seed Produced: Germination Percent 1986
5	6	5.8	10.3	2.00	6651	6061	1963	2475	23.5	23.0
9	6	4.4	4.0	0.21	631	1026	53	486	33.8	23.7
24	8	9.1	13.7	1.82	6886	9437	1373	3117	58.5	62.0
39	6	5.0	6.9	0.29	851	1840	25	736	57.8	59.7
42	6	6.2	7.3	1.00	3036	2748	546	1388	59.5	73.7
46	6	6.6	8.0	0.52	2067	1361	466	420	53.3	50.7
53	6	7.4	11.0	1.33	4788	7035	1204	3071	49.3	55.7
					Total Seed Yield		5630g	11693g		

The future demand for improved black alder seed is hard to predict because it depends on the rise in interest in establishing energy plantations and/or other uses of fast-growing hardwoods. We asked the nurserymen of nine

midwestern state forestry organizations to project seedling demand as they saw it developing in their states. Production estimates ranged from 0 to 90,000 seedlings/year. If we compare black alder to species with similar uses that are already established in the commercial trade, 70,000 seedlings/year seems to be an appropriate level of production (Scholtes 1985). Schoepmeyer (1974) estimated that 10,000 nursery seedlings could be produced per pound of seed. Hence, an entire state's planting needs could be supplied by 3 seed orchard trees. More likely, several adjacent states with similar planting environments could share a small orchard, and the minimal number of trees needed to provide genetic diversity would be more than enough trees to supply the need for seed.

Establishment

The best time of year to move selected trees with a tree spade is in late fall. Black alder flowers are receptive in late February to early March (in central Iowa), so trees must be moved in the fall to assure that the first season's seed production is based on the pollen of the orchard trees. Spring is also a period of very poor trafficability on the bottomland soils where alder plantations are typically located. We tried to move our trees in the spring of 1984, but excessively wet conditions prevented the work until July of that year. Then, the trees had only a brief period of good establishment conditions before an August drought caused substantial top dieback and mortality in five of our selections and led to an invasion of wood-boring insects. In 1986, a series of windstorms broke the tops out of all the large stems in the orchard, leaving only coppice growth. All selections should be cloned by the rooting of cuttings before they are moved as insurance against transplant shock mortality.

Rooted cuttings of black alder can be produced by taking the most actively growing new branch growth available on the selections made in the field. The field cuttings are taken early in the morning or during rainy weather when internal moisture stresses are least. The cuttings are transported on ice back to a greenhouse where single internode cuttings are taken from the semilignified portion of the current season's growth The very succulent branch tips are not used. The cuttings are given a 5-sec basal dip in 5,000 ppm IBA and are stuck in a rooting media (equal parts peat, vermiculite, and pearlite) under intermittent mist. After about 3 weeks of rooting time, the cuttings are transferred to pots or other containers to be grown to at least 45 cm in height before they are planted in the field. Rooting of cuttings from the field usually succeeds at no better than a 50% rate, so that must be taken into consideration during scion collection.

A seed orchard spacing of 5x5 m with the trees staggered between rows works well for the size of tree we want to use. Typically, a 500-ft isolation zone is left around seed orchards to minimize contamination from nonselected pollen sources (Wright 1976). However, that design feature is based on pollen dispersal studies with conifers; effective pollen dispersion distances of hardwoods are not well documented. Wright (1976) did report that, for American elm, an isolation distance of 2200 ft would be necessary to exclude 91% of the contaminating pollen. We left 635 m (2080 ft) of isolation zone in establishing our black alder seed orchard. When we quantified pollen

dispersion in the spring of 1987 (figure 1), contamination was much greater than we expected. One standard deviation for the dispersal curve extends out to 710 m (2329 ft). Pollen contamination at the seed orchard site was approximately 26% of the source frequency. The terrain in the vicinity of our Rhodes Farm alder plantings consists of a north-south valley surrounded by steep, wooded hillsides that may funnel more pollen from our provenance and clonal tests to our seed orchard than normally would be the case. However, to be safe, the isolation zones for black alder seed orchards should be larger than we had previously recommended. Because tree alders are exotics in most of eastern North America, it should be less of a problem to find a suitably isolated orchard site than is the case for most other important tree species.

Management

The harvesting of black alder strobili has typically been scheduled for late fall (Schoepmeyer 1974) after the scales begin to reflex. We initiated a study of seed maturity dates as an aid to our breeding program. Seed viability reaches its maximum as early as the third week in August (figure 2), when the strobili are still very green. Pizelle (1984) reported a similar finding with black alder grown in France. However, seed extraction is greatly facilitated if strobili have just begun to turn brown (about the third week in September). It definitely is not necessary to wait for the strobili scales to begin opening before the crop is harvested. Brown strobili will open and release their seed easily after a few days of air-drying.

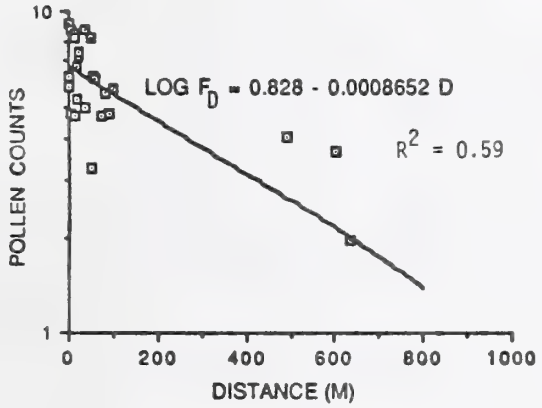


Figure 1. Pollen counts at various distances from black alder stands at the Rhodes Farm. Note that the ordinate axis is logrithmic. F = pollen frequency at distance = D.

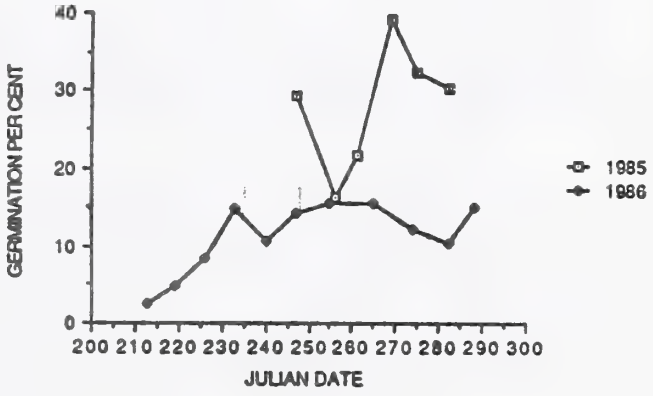


Figure 2. Average germination percent of seeds of three black alder clones from strobili harvested in 1985 and 1986 at the Rhodes Farm.

Although we have not yet run controlled experiments, it does seem that it will be very beneficial to have a means to irrigate the orchard trees during periods of moisture stress. The faster trees can be grown vegetatively, the sooner they reach a flowering condition and the more seed they produce. There does not seem to be a flower-inducing effect of moisture stress as there is in some other tree species. However, the most important concern is likely to be the impact on seed quality. Black alder has delayed fertilization; pollination takes place in early spring. After an initial period of pollen tube growth, there is a hiatus while female meiosis occurs, and then

fertilization is completed at the beginning of the summer (McVean 1955). We have observed that our black alder trees have produced strobili and seed every year once they begin flowering, but filled seed percentage can be very low (including 0%) in years when we have a dry May/June period. The availability of irrigation water at that time may be critical to the success of a seed orchard.

No serious strobili or seed pests have been encountered yet. We have had serious infestations of the European alder leafminer, Fenusa dohrnii (Tischbein), in all our plantings. We are in the middle of a multiple-year study of the growth impact of this defoliating insect, but it seems that a range of 15 to 40% dry-weight reduction is occurring, depending on other growing conditions. We have started a program to select and breed for resistance, but in the meantime, we have determined that the application of DiSyston 15 G systemic insecticide will control the problem. We are now annually applying 40 g around the base of each seed orchard tree and covering it with mulch. In a mature seed orchard, we would need 300-600 g of DiSyston per tree. It is likely that this systemic treatment will also help control insects that might attack the strobili.

The early and abundant production of seed, combined with the strong coppicing ability of black alder affords the opportunity for unusual approaches to seed-orchard management. We suggest that orchards be twice as large as necessary to meet seed demands. Then, half of an orchard can be cut every 5 years, any clones that had fallen into disfavor could be replaced, and the stand allowed to regrow for 10 years. After the first or second year, the sprout clumps can be thinned back to one or a few main stems for each tree. By 5 years of coppice age, seed production should be abundant again. This rejuvenated portion of the orchard can then assume the mission for the other half of the orchard where the trees are getting unmanageably large.

Alternatively, it may be possible to carry six to eight main sprout stems on each orchard stump and harvest one each year for easy seed collection. That stem would then give rise to coppice sprouts that should be thinned back to the best replacement stem after 1 or 2 years. We are just beginning to test this alternative. In conclusion, we believe the characteristics of alder allow for the use of small seed orchards that can be modified over time to keep producing the best seed available based on research as it is completed.

LITERATURE CITED

Faulkner, R. (ed.) 1975. Seed Orchards. Br. For. Comm. Bull. 54. Her Magesty's Stationery Office, London.

Hagman, M. 1975. Incompatability in forest trees. Proc. R. Soc. Lond. Ser. B. Biol. 188:313-326.

Hall, R. B., and C. A. Maynard. 1979. Considerations in the genetic improvement of alder. Proc. Workshop on Symbiotic Nitrogen Fixation in the Management of Temperate Forests. Oregon State Univ., Corvallis, Oregon. pp. 322-344.

Hall, R. B. and G. A. Miller. 1983. Selection and breeding strategy for an exotic species: Alnus glutinosa in North America. Proc. 3rd North Cent. Tree Impr. Conf. pp. 233-244.

Hall, R. B., G. A. Miller, T. L. Robison and O. U. Onokpise. 1983. Developing Alnus for use in intensive culture. In: (Hansen, E. A., Compiler) Intensive Plantation Culture: 12 Years Research. U. S. For. Serv. Gen. Tech. Rep. NC-91. pp. 35-45.

Hoekstra, P. E. 1965. A simple inexpensive pollen trap. U. S. For. Serv. Res. Note SE-40.

Maynard, C. A. and R. B. Hall. 1980. Early results of a range-wide provenance trial of Alnus glutinosa (L.) Gaertn. Proc. 27th Northeast. For. Tree Impr. Conf. pp. 184-201.

McVean, D. N. 1955. Ecology of Alnus glutinosa. I. Fruit formation. J. Ecol. 43:46-60.

Oak, S. W., and R. D. Dorset. 1983. Phomopsis canker of European black alder found in Kentucky seed-production areas. Plant Disease 67:691-693.

Pizelle, G. 1984. Seasonal variations of the sexual reproductive growth and nitrogenase activity (C_2H_2) in mature Alnus glutinosa. Plant Soil 78:181-188.

Ranney, J. W., L. L. Wright, J. L. Trimble, R. D. Perlack, D. H. Dawson, C. R. Wenzel and D. T. Curtin. 1985. Short Rotation Woody Crops Program: Annual Progress Report for 1984. ORNL-6160. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Robison, T. L. and R. B. Hall. 1981. Approaches to European alder improvement. Proc. 2nd North Cent. Tree Impr. Conf. pp. 65-77.

Schoepmeyer, C. S. 1974. Alnus B. Ehrh. Alder. In: Seeds of Woody Plants in the United States. U.S. Dep. Agric., Agric. Handb. 450:206-211.

Scholtes, J. R. 1985. 1985 Northeastern Area state owned nursery production. USDA Forest Service State & Private Northeastern Area Report.

Wright, J. W. 1976. Introduction to Forest Genetics. Academic Press. New York.

EFFECTS OF RELATED AND UNRELATED GRAFT PARTNERS IN SLASH PINE (*Pinus elliottii* Engelm.)

R.C. Bower[1/] and C.R. McKinley[2/]

Abstract.--The paper presents the results of a study designed to test the effect of degree of relationship between scion and rootstock on the survival, height growth and incompatibility of slash pine grafts. After four years, no significant gain was made in survival, height growth or reduction of incompatibility by using rootstocks that were related to the scions to which they were grafted.

Additional keywords: Stock-scion relations, graft incompatibility, vegetative propagation.

In a comprehensive review of scion and rootstock relationships in woody perennials, Tubbs (1973) cited many examples of the use of genetically identified rootstocks to influence scion performance and to overcome some of the problems of grafting in these species. Lantz (1970) suggested that such an approach might be worthwhile in tree improvement programs, particularly in those cases where graft incompatibility is a significant problem.

Several authors have reported decreases in incompatibility of forest trees through the use of rootstocks that were related to the scion material (Copes 1973, Denison 1973, Slee and Spidy 1970, van der Sijde 1974).

Rootstocks of known genetic origin have also been shown to influence the growth of scion material in a variety of species (Schmidtling 1973, McKinley 1975, Melchoir 1984). The objective of this study was to determine if rootstocks that were related to the scions improved the survival and growth or reduced the incompatibility of slash pine scion material.

MATERIALS AND METHODS

Seeds were collected from a total of 26 open- and control-pollinated families in a Texas Forest Service slash pine seed orchard. Seeds were obtained with the following parentages: open-pollinated with the female parent being an incompatible clone, control-pollinated with either the male or the female parent being an incompatible, and control pollinated with both parents compatibles. Incompatible clones are identified as those clones in which certain individual ramets demonstrated definite symptoms of incompatibility such as scion overgrowth, stunted growth, and chlorotic needles.

1/ Forest Geneticist, MacMillan Bloedel Limited, Nanaimo, B.C.

2/ Assistant Professor, Texas A & M University, College Station, Texas. The authors wish to express appreciation to the Texas Forest Service for supporting this project.

Seeds were germinated in sand and allowed to grow for two months in greenhouse conditions. Approximately 12 seedlings from each family were transplanted into three-inch peat pots and moved to a lathhouse.

Grafting was done at the beginning of the second growing season (February-March). All grafts were made by removing the top one-third of the seedling, then grafting this "scion" onto the desired rootstock with a cleft graft. By switching the scion material from two separate plants, the same genotypes could be used in two graft combinations; each as a scion and each as a rootstock. Grafts involving the same genotype (autoplastic) were made by removing the scion as above and grafting directly back onto the remaining stem. Table 1 lists the different relationships between the scion and rootstock which were utilized.

Table 1.--Relationship classes of grafts made in this study

Graft Partners Related:

1. Rootstock and scion consist of the same genotype (autoplastic).

   Graft made by removing the top of the seedling and then grafting it back onto the remaining part of the plant.

2. Rootstock and scion related as full-sibs.

   Full sib members of control-pollinated families utilized as both rootstock and scion material.

3. Rootstock and scion related as half-sibs.

   Scion material obtained from open-pollinated families and grafted back onto rootstocks of the same families.

Graft Partners Unrelated:

1. Scion material switched between open-pollinated families which had incompatible clones as female parents.

2. Scion material switched between control-pollinated sources which had incompatible clones as either male or female parent.

3. Scion material switched between control-pollinated sources which had compatible clones as both parents.

The grafts were maintained in the lathhouse for one year. The surviving grafts were then outplanted in a field planting using a completely random design.

Total height, survival and incompatibility were assessed at the end of each growing season for four years. Lantz's (1973) definition of graft incompatibility was used. He defined graft incompatibility as a stock-scion interaction occurring in a successfully established graft which causes a decline in vigor of either stock, scion or both. This paper presents the results at the end of year four.

RESULTS AND DISCUSSION

Statistical analyses were performed to test related versus unrelated combinations, degree of relatedness within related combinations and compatibility of parents within unrelated combinations. To compensate for differences in the initial height of the grafts, height growth was used in the analyses. Height growth was calculated as the difference between height for year four and the initial height.

Family differences were not analyzed in this study. Several families had too few seedlings to allow grafts to be made in enough combinations to make a valid test. All graft combinations within a relationship class were treated as individual observations without regard to family designation. It is known that specific scion-rootstock combinations can have a significant effect on survival, incompatibility and growth (McKinley 1974, Bower 1977). However, it was felt that the large number of observations within a relationship class would give a reasonable average value for the relationship class.

Mean survival, height growth and incompatibility by relationship class are presented in Table 2. For ease of discussion, all analyses for a specific class will be presented together.

Table 2.--Mean fourth-year survival, growth and incompatibility by relationship class

Relationship Class	Survival (%)	Growth (cm)	Incompatibility (%)
Related	75	237	13
Self	86a	243a	3a
Full-sib	72b	238a	19b
Half-sib	65b	229a	18b
Unrelated	69	228	15
Both parents incompatible	60a	224a	20a
One parent incompatible	78b	215a	11a
Neither parent incompatible	82b	243a	9a

Means followed by the same letter are not different at the 0.05 level of probability.

RELATED VERSUS UNRELATED

Analysis of variance showed no significant differences in survival, height growth or incompatibility between the related and unrelated combinations.

GROUPS WITHIN RELATED COMBINATIONS

Analysis of variance showed highly significant (0.01) differences for survival, no differences for height growth and significant (0.05) differences for incompatibility among the three related groups. The autoplastic grafts had the highest survival and lowest incompatibility. There were no differences between the grafts that were related as half-sibs and those that were related as full-sibs for survival or incompatibility.

Three of the autoplastic grafts were classified incompatible in year three, based on short chlorotic needles. These three grafts were dead in year four. No evidence of scion overgrowth was observed. Since the scion and rootstock of these grafts were of identical genotypes, an incompatible reaction would not be expected.

Three types of material were used in making the autoplastic grafts: (1) seed collected from incompatible ramets of incompatible clones, (2) seed collected from compatible ramets of incompatible clones, and (3) seed collected from controlled pollinations in which one parent was an incompatible clone. It is notable that the three grafts classified as incompatible were all from families in which the seed had been collected from incompatible ramets of incompatible clones. The results of a chi-square analysis showed that the three incompatible grafts could have occurred in this category purely by chance. Unless there is evidence of scion overgrowth, classifying a graft as incompatible is somewhat subjective, therefore, these grafts may have been incorrectly classified.

GROUPS WITHIN UNRELATED COMBINATIONS

Significant (0.05) differences in survival were observed among groups within the unrelated category. Graft combinations in which both the scion and the rootstock were from compatible sources had the highest survival. Those graft combinations in which both the scion and rootstock were from incompatible sources had the poorest survival.

The analysis of variance for height and incompatibility showed no significant differences.

Since all unrelated grafts were made in reciprocal form, an analysis was performed to determine if any groups performed differently used as rootstocks or used as scions. No significant differences were observed in survival, incompatibility or scion growth among groups of reciprocal grafts.

CONCLUSIONS

After four years, no significant gain was made in survival, percentage of incompatibility or height growth by using rootstocks that were related to the scions to which they were grafted. The related combinations tended to have

better survival, a lower percentage of incompatibility and more height growth than the unrelated combinations. However, further analyses showed this trend to be the result of the autoplastic grafts.

Among unrelated combinations, there were significant differences in survival. Those grafts in which both the scion and rootstock were from incompatible sources had the poorest survival. Those grafts in which both the scion and rootstock were from compatible sources had the highest survival. Although the differences were not significant, those grafts in which both the scion and stock were from compatible sources had less incompatibility than those in which both scion and rootstock were from incompatible sources.

No significant differences were seen in scion growth among the various unrelated combinations.

The results of this study are consistent with Schmidtling's (1986) conclusion that for slash pine, grafting clones onto related rootstocks would be a wasted effort for most clones.

LITERATURE CITED

Bower, R.C. 1977. Graft incompatibility and scion-rootstock relationships in slash pine (Pinus elliottii Engelm.) and loblolly pine (Pinus taeda L.). Ph.D. thesis, Texas A & M Univ., College Station, Tx. 70 p.

Copes, D.L. 1973. Inheritance of graft compatibility in Douglas-Fir. Bot. Gaz. 134(1):49-52.

Denison, N.P. 1973. Selection and breeding to improve some tropical conifers, (eds J. Burley and D.G. Nikles). Vol. 2, 277-284. Oxford; Commonwealth Forestry Institute.

Lantz, C.W. 1970. Graft incompatibility in loblolly pine. Ph.D. thesis, North Carolina State Univ., Raleigh, N.C. 102 pp. (University Microfilms Ann Arbor, Mich.)

Lantz, C.W. 1973. Survey of graft incompatibility in loblolly pine. In: Proc. 12th South. For. Tree Imp. Conf.; 1973 June 12-13; Baton Rouge, L.A. Louisiana State University. pp 86-90.

McKinley, C.R. 1974. Stock-scion relationships and graft incompatibility in slash pine (Pinus elliotti Engelm.) and loblolly pine (Pinus taeda L.). Ph.D. thesis, Texas A & M Univ. College Station, TX. 88 p.

McKinley, C.R. 1975. Growth of loblolly scion material on rootstocks of known genetic origin. In Proc. 13th South. For. Tree Imp. Conf. Raleigh, N.C. pp 230-233.

Melchoir, G.H. 1984. The influence of defined rootstocks on graphs of Norway spruce (Pinea abies L. Karst). Silvae Genet. 33(1):28-32.

Schmidtling, R.C. 1973. Rootstock influences early fruitfulness, growth and survival in loblolly pine grafts. In: Proc. 12th South. For. Tree Imp. Conf.; 1973 June 12-13; Baton Rouge, L.A. Louisiana State University. pp 79-85.

Schmidtling, R.C. 1986. The effect of genetically related rootstock on survival, growth, and flowering in grafted slash pines. In: IUFRO Con. Proc., Williamsburg, VA. pp 422-428.

Slee, M.U. and T. Spidy. 1970. The incidence of graft incompatibility with related stock in Pinus caribea Mor. var. hondurensis B. et G. Silvae Genet. 19(5-6):184-187.

Tubbs, F.R. 1973. Research fields in the interaction of rootstocks and scions in woody perennials — Part 1. Hort. Abs. 43(5):247-253.

DESIGN AND MANAGEMENT OF PROGENY TESTS

MODERATED BY DR. PETER FERET

Virginia Polytechnic Institute

PRACTICAL USES OF BREEDING VALUES IN TREE IMPROVEMENT PROGRAMS AND THEIR PREDICTION FROM PROGENY TEST DATA

T. L. White and G. R. Hodge[1]

Abstract.--One of the main functions of genetic tests is progeny testing, that is prediction of breeding values of parents based on performance of their offspring. By definition, parents with high breeding values tend to produce offspring with superior performance characteristics. Breeding values for a specific trait are unobservable random variables that can never be known exactly. However, because the information is useful in so many ways in tree improvement programs, it is critical that they be predicted both accurately and precisely.

This paper is divided into three major sections. First, we highlight how breeding value predictions for all parents (selections) can be used at many stages of a program to increase genetic gain. Second, several factors are described that make it difficult to precisely predict breeding values (i.e. rank parents) from progeny test data: tests of different ages, tests of different precisions, parents represented in different number of tests. Finally, an analytical methodology called Best Linear Prediction is discussed which was developed by dairy breeders specifically to deal with "messy" progeny test data. This method and a related method (called Best Linear Unbiased Prediction) may well have a place in tree improvement programs that need to efficiently rank parents based on multiple sources of "messy" data.

Additional Keywords: Best Linear Prediction, Best Linear Unbiased Prediction, Messy Data, slash pine, selection index.

INTRODUCTION

Genetic tests are widely used in tree improvement programs for a variety of reasons (see Libby 1973, McKinley 1983). One important function of genetic tests is progeny testing: determining the values of parents based on the performances of their offspring (Allard 1960, p470). For example, the performances of open-pollinated offspring from first-generation selections planted in randomized, replicated field tests can be used to rank the selections. Selections whose offspring perform well for a specific trait, such as disease resistance, are predicted to have high breeding values for that trait. Breeding values are best thought of as unobservable random variables whose values can never be known exactly, but only predicted (Henderson 1973, 1977, 1984 p. 38). That is, based on an observed sample of offspring from a given parent (i.e. a selection) growing in progeny tests, we wish to predict

---

[1]/ Director and Geneticist, Cooperative Forest Genetics Research Program, Department of Forestry, University of Florida, Gainesville, FL, 32611.

how future offspring will perform under operational conditions (i.e. on different field sites and possibly with different cultural treatments than the progeny tests received).

Precise and accurate predictions of breeding values for all selections in a tree improvement program are essential for maximizing genetic gain; however, the "messy" nature of progeny test data from many programs complicates the analytical procedures required to develop these predictions. In this paper, we first highlight the many ways that accurate, precise predictions of breeding values can be used to further tree improvement programs. This is followed by a description of the types of "messy" data problems that arise and complicate the prediction of breeding values from progeny tests. Finally, an analytical method is discussed which has been dveloped to predict breeding values from "messy" progeny test data. Examples are given from a slash pine breeding program to compare the new method to one currently in use.

PRACTICAL USES OF BREEDING VALUES IN TREE IMPROVEMENT PROGRAMS

The precise, accurate predictions of breeding values are critical in tree improvement programs because the predicted values are used in many ways to increase genetic gains and further the breeding program (see McKinley 1983, Lindgren 1986, White 1987). The brief description of these uses (below) focuses on programs working with sexually-propagated species, though the concepts also apply to vegetatively-propagated species (much of this is adapted from White 1987).

Production Population

The production population in any given generation of breeding is that group of selections used to produce propagules for operational forestation. Clonal (grafted) seed orchards are the most common type of production populations in first-generation tree improvement programs. Increased yield and product quality from the harvesting of these improved forests are the primary realized benefits of most tree improvement programs. Once selections have been ranked based on their predicted breeding values, the production population can be managed to achieve maximum genetic gains.

1. Upgrading the Production Population: Low ranking members can be excluded from the production population and therefore not allowed to contribute genes to operational plantings. As examples, inferior clones can be rogued from first-generation orchards and/or mid-generation orchards (i.e 1.5 generation orchards) can be established which include only superior genotypes.

2. Tailoring Deployment Strategies: The predicted breeding values can also be used to enhance deployment of seed (or vegetative propagules) to specific planting sites. Many companies in the Southeast collect orchard seed and keep it in separate categories for deployment. For example, seed from clones known to be resistant to fusiform rust can be deployed to planting sites expected to experience a high level of exposure to the disease. Deployment strategies can become quite complex and may involve greater use of seed from clones with high predicted breeding values (Lindgren 1986).

Breeding Population

1. Upgrading the Breeding Population: If the intermating of selections is delayed until after progeny test results are available, low ranking selections can be excluded from the breeding population. This increases the genetic quality of the base population of offspring from the intermating, because superior genotypes contribute a higher proportion of the alleles. This potential increase in gain has to be weighed against the time delay of waiting for the progeny test results and the reduction in effective population size.

2. Enhancing Mating Designs: Predicted breeding values can be used to create more efficient mating designs. As only one example (see White 1987), parents with high predicted breeding values can be used in more crosses (Lindgren 1986).

Advanced Generations

1. Increasing Gain from Selection: Advanced-generation selections will typically be made in the pedigreed base populations generated by intermating the members of the breeding population. The predicted breeding values will be used as parental information to aid in finding the best families within which to make selections.

2. Other Uses: The predicted breeding values of the selections of a previous generation of breeding can continue to serve as ancestral information to be combined with the data obtained from the testing the current generation's selections. This enhances gain by increasing the precision of the current breeding value predictions.

MESSY DATA PROBLEMS IN BREEDING VALUE PREDICTION

Progeny tests are usually replicated across both years and diverse planting locations, but because of space limitations in any one field site, all selections are usually not represented in any given test. Data must be combined across multiple tests to develop breeding value predictions and some of the potential factors which can complicate this data analysis are briefly summarized below (see White et al 1986 for more details).

Test Age

Sometimes, tests are established over a wide range of years and test data ranging from say 3 to 15 years old must be combined into a single breeding value prediction for each trait (such as volume growth) for each selection. For growth traits, the older data will probably be more reliable in predicting relative growth performance at harvest (Lambeth 1980) and should therefore receive more weight in the prediction process. This may differ for different traits.

Test Precision

Family means (and rankings) vary in precision from test to test. Tests with small numbers of blocks, variable environmental and vegetation conditions, poor survival, etc, will be less effective in ranking families

based on genetic values. In these "poor" tests, more of the variablility in family means is attributable to spurious environmental effects and, intuitively, it seems they should receive less weight in the development of breeding value predictions.

Parents in Different Numbers of Tests

If parents are represented in different numbers of tests and are ranked by the grand family means calculated across all tests in which each occurs, parents with the highest and lowest ranks will tend to be those in the fewest tests (White et al. 1986). This occurs because family means based on many tests will have a lower spread (variance) than those in few tests. This "variance" problem is well known in dairy breeding (Henderson 1973) and results in ranking errors since too many of the high ranking parents will tend to be the least tested (i.e. in fewer tests).

BEST LINEAR PREDICTION OF BREEDING VALUES

Most approaches to the analysis of "messy" progeny test data in forestry (Hatcher et al. 1981, Cotterill et al. 1983) treat family means as fixed effects (Class I model of Eisenhart 1947) and aim at developing family mean estimates of which are adjusted to be free of the complicating factors described above. An alternative analytical approach, developed by Henderson (1973, 1977, 1984), treats parental breeding values as unobservable random effects to be predicted from the observed sample of offspring in progeny tests (Class II model of Eisenhart 1947). This approach leads to two analytical methodologies, Best Linear Prediction (BLP) and Best Linear Unbiased Prediction, the latter of which is used extensively to predict breeding values in animal science. We believe that both of these methods can be useful in predicting breeding values from messy progeny test data in forestry. Both methods involve similar logic and rationale; here we first briefly describe the principles of BLP (see White et al. 1986 for a more complete description) and then summarize its application to open-pollinated slash pine progeny tests.

Properties of BLP

The formula for calculating Best Linear Predictions is (see Henderson 1973, 1977, 1984 for derivation):

$$\hat{\mathbf{g}} = \mathbf{C}'\mathbf{V}^{-1}(\mathbf{y}-\boldsymbol{\alpha}).$$

Here, $\mathbf{y}$ is an nx1 vector of observed data (e.g.,family means from open-pollinated progeny tests) being used to predict $\mathbf{g}$, a px1 vector of breeding values for the p parents represented in the tests. $\mathbf{C}$ is cov($\mathbf{y},\mathbf{g}'$), an nxp matrix of genetic covariances between the observed data and the breeding values being predicted (for example, the covariance between an observed family mean for volume at 5 years with the breeding value of the parent for volume production at maturity). $\mathbf{V}$ is var($\mathbf{y}$), an nxn matrix of variances and covariances among the observations (e.g., the variance of family means in a given test and the covariances between family means in different tests).

Finally, $(\mathbf{y}-\boldsymbol{\alpha})$ expresses the data, $\mathbf{y}$, as deviations from their means, $\boldsymbol{\alpha}$ (e.g., family means from a given test expressed as deviations from the overall test mean). Predictions from this approach have many desirable properties if $\mathbf{C}$, $\mathbf{V}$, and $\boldsymbol{\alpha}$ are known or well-estimated (see Henderson's work).

BLP uses observed data from any ages and traits to predict the breeding values for the traits of interest. This applies to a wide variety of cases including prediction of multiple traits (e.g. volume growth at maturity and rust resistance), different types of relatives, data of different ages and precision and prediction of traits not observed directly (indirect prediction). BLP is an application of selection index (Hazel 1943) that develops a different set of index coefficients for each parent. $\mathbf{C}'\mathbf{V}^{-1}$ can be thought of as developing multiple regression coefficients relating $\mathbf{y}$ to $\mathbf{g}$ (Thompson 1979).

The set of coefficients developed for a particular parent reflects the quality (age, precision) and quantity of data observed for that parent. Predicted breeding values will tend to spread out more (have larger variances among predictions) when there are lots of observed data of high precision that are closely correlated to the trait being predicted. Conversely, if the data are sparse, imprecise and poorly correlated with the trait being predicted (such as using juvenile progeny test volumes to predict mature volume breeding values), the predicted breeding values (of the mature trait being predicted) will cluster more closely around zero. Heuristically, if the deviations of observed data about their means, $(\mathbf{y}-\boldsymbol{\alpha})$, mostly reflect environmental as opposed to genetic causes and are not correlated to the trait being predicted, then they are shrunken (regressed) back towards the mean farther. BLP weights each observed family mean according to its precision and degree of correlation with the trait being predicted and thus handles all of the "messy" data problems described above in one step.

Application of BLP to Slash Pine Open-Pollinated Progeny Test Data

The Cooperative Forest Genetics Research Program (CFGRP) began in the mid-1950's with the mass selection of superior slash pine phenotypes from natural stands and plantations (Goddard 1981). Each cooperating organization made 50-200 selections (cooperative-wide total ≈ 1500) which were immediately grafted into clonal seed orchards for the production of improved seed. As the the orchards began to produce seed, cooperators used this orchard open-pollinated seed to establish progeny tests of their selections. Tests were established over a long period of time (1963 to 1983) and to date over 362 tests have been established. Test designs vary, but an average test is a randomized complete block containing 30 families and 6 blocks with a seven to ten-tree row-plot representing each family in each block. Each tree in a test is measured at 5, 10, and sometimes 15 years old for rust incidence and volume. Any given parent is represented in from 1 to 22 such tests.

The current analytical method for analyzing these progeny test data calculates standardized average performances for each test and these are averaged over all tests in which a parent occurs (see Cotterill et al. 1983, Hatcher et al. 1981 for similar approaches). The standardized scores are expressed in standard deviation units from the mean; for example, a parent which was consistently superior for growth over several tests might have a score of 2 while a poor parent would be, say -2. These standard scores have been used to rank parents for the purposes of rogueing orchards,etc.

For BLP, breeding values to be predicted were rust incidence in a 50% rust incidence environment and volume growth at 15 years (White and Hodge 1987 present complete details of this BLP application). These roughly correspond to the rust and growth standard scores, but breeding values are always expressed in the units of measurement as deviations from the average. Thus parents with rust breeding values of say 20% (in a fifty percent hazard environment) and say 1.5 cubic feet (a deviation of 36% above the average of 4.21 cu ft. in 15 year old progeny tests) would be good.

A subset of 28 tests with data for both volume and fusiform rust incidence at all three measurement ages (5, 10 and 15 yrs) was used to estimate variances and covariances for the **C** and **V** matrices . Both variances and covariances varied dramatically from test to test and predictive regression models were developed to apply to test data not in the subset of 28. For rust, data from tests with very low or very high rust incidence levels had smaller variances of family means and smaller covariances with the trait being predicted (i.e. rust incidence in a 50% hazard environment). For growth, family means from young tests (especially 5 yrs old) and from tests with high coefficients of variation (large experimental error) had higher variances and smaller covariances with 15 year volume. Data from these types of tests get less weight and result in breeding values that are more "shrunken" or "regressed" back towards the mean.

To apply the formula for BLP, family means were calculated for each test and expressed as deviations from the estimated test mean (both checks and family means were used to estimate test means). Preliminary Best Linear Predictions of breeding values for rust resistance and volume at 15 years were then calculated for approximately 1300 parents and compared to the corresponding standard scores currently used. The coefficients of determination between standard scores and BLP breeding values were $r^2 = 0.77$ and $r^2 = 0.52$ for rust and volume, respectively. Also, for rust, the average absolute rank change from standard scores to BLP was approximately ±100, while for growth it was ±200. So based on these two measures of comparison, rankings of the two analytical systems are more similar for rust than for growth. Presumably, the fact that rust resistance is more heritable than growth and that the data are not so dependent upon measurement age makes the rust data less "messy" and therefore less benefit accrues from the use of BLP. For growth, large rank changes between the two systems occurred in certain situations. For example, a parent ranked high under the old system of standard scores falls considerably in the ranks of the BLP system if its progeny test data are from only a few, young, imprecise tests.

As described in the section on data problems, the standard score approach results in a tendency for parents in fewer tests to be at the extremes of the rankings. BLP has just the opposite trend. On the average in our slash pine data base, parents are in approximately 4 tests in which volume was measured. However, if the top 1% of the parents are selected to be used say in an orchard, those 13 parents (out of ≈ 1300) are represented in an average of approximately 2.5 tests vs. 6 tests using standard scores vs. BLP. Thus, using the standard score approach, there is a tendency to choose as the better parents those that are in fewer tests, while with BLP the tendency is just the opposite: if a parent is good in several tests, it is predicted to have a high breeding value.

DISCUSSION

Best Linear Prediction treats progeny test data in an intrinsically different fashion than do most methods based on averaging family means or scores over tests. For data which is not very messy (tests of nearly the same precision, tests of similar ages, and all parents in similar or large numbers of tests), the benefits of BLP will not be of any practical importance. That is, rankings from most approaches will be similar. However, whenever data from a wide variety of sources (varying ages or precisions, different types of designs or relatives, greenhouse screening data, etc) need to be aggregated to rank parents, BLP (and/or BLUP) may offer significant advantages. For instance, in advanced generation breeding programs, it may be necessary to use data from several generations of testing to precisely predict breeding values. One distinct benefit of BLP is the ease in which multiple traits are handled. Multiple trait selection indices are easily formed after or at the same time as the predictions are made for the individual traits.

The accuracy and precision of BLP breeding values depend on the estimation of the variances and covariances in the **C** and **V** matrices. While more work is needed to assess the sensitivity of the predictions to errors in estimating these second moments, we currently believe that for most tree improvement programs they can be estimated well enough to make BLP or BLUP a substantial improvement over conventional methods of analyzing "messy" progeny test data.

LITERATURE CITED

Allard, R.W. 1960. Principles of Plant Breeding.John Wiley and Sons, New York,NY. 485p.

Cotterill, P.P., R.L. Correll and R. Boardman. 1983. Methods of estimating the average performance of families across incomplete open-pollinated progeny tests. Silvae Genet 32:28-32.

Eisenhart, C. 1947. The assumptions underlying the analysis of variance. Biometrics 3:1-21.

Goddard, R.E. 1980. The University of Florida Cooperative Forest Genetics Research Program. pp. 31-42 In Proc. 15th North Amer. Quant. For. Genet. Group. August 6-8. Coeur d'Alene, ID.

Hatcher, A.V., F.E. Bridgwater and R.J. Weir. 1981. Performance level-Standardized score for progeny test performance. Silvae Genet 30:184-187.

Hazel, L.N. 1943. The genetic basis for constructing selection indexes. Genetics 28:476-490.

Henderson, C.R. 1973. Sire evaluation and genetic trends. pp. 10-41. In Animal Breeding and Genetics Symposium in Honor of J. Lush. Animal Sci. Assoc. Amer., Champaign, Ill.

Henderson, C.R. 1977. Prediction of future records. pp. 615-638. In Proc. Internat. Conf. Quan. Genet.(E. Pollack, O. Kempthorne and T. Bailey eds.). The Iowa State Univ. Press, Ames, IO.

Henderson, C.R. 1984. Applications of Linear Models in Anumal Breeding. Univ. Guelph Press. Guelph, Ontario, Can. 462p.

Lambeth, C.C. 1980. Juvenile-mature correlations in Pinaceae and implications for early selection. Forest Sci 26:571-580.

Libby, W.J. 1973. Domestication strategies for forest trees. Can J For Res 3:265-276.

Lindgren, D. 1986. How should breeders respond to breeding values?. pp. 361-372. In: Proc. IUFRO Conf. on Breeding Theory, Progeny Testing and Seed Orchards. October 13-17, Williamsburg, VA.

McKinley, C.R. 1983. Objectives of progeny tests. pp. 2-13. In Progeny Testing of Forest Trees. Southern Coop. Series Bull. 275. Texas A & M Univ., College Station, TX, 77843.

Thompson, R. 1979. Sire evaluation. Biometrics 35:339-353.

White, T.L. 1987. A conceptual framework for tree improvement programs. In Preparation.

White, T.L. and G.R. Hodge. 1987. Best linear prediction of breeding values in a slash pine tree improvement program. In Preparation.

White, T.L., G.R. Hodge and M.A. Delorenzo. 1986. Best linear prediction of breeding values in forest tree improvement. pp. 99-122 In Southern Coop. Series Bull. 324. Univ. of Florida, Gainesville, FL.

RELATIONSHIP BETWEEN STABILITY INDICES AT THE TIME OF GERMINATION AND AT THE SEEDLING STAGE IN JACK PINE

D. R. Govindaraju,[1] and B. P. Dancik [2]

Abstract.--The predictability of stability indices at different developmental stages of seedling growth was studied in forty open-pollinated families of jack pine (Pinus banksiana Lamb.). Two sets of studies were conducted simultaneously under greenhouse conditions. In the first, the seeds were germinated under four levels of water stresses; in the second, they were grown on four soil media. Stability indices were calculated for radicle length of germinating seeds and total seedling biomass using the Eberhart-Russell model. The correlation of stability indices from the two developmental stages under similar environmental conditions was insignificant. We conclude that the unpredictability of stability may be associated not only with genotype-environment interactions, but also with developmental and/or demographic genetic effects.

Additional keywords: Jack pine, stability indices, developmental stages.

Breeding for consistent performance of genotypes across environments is one of the primary goals in many tree improvement programs. The relative sensitivity or stability of genotypes across environments is described in terms of genotype-environment interactions (Haldane 1946, Falconer 1952, Allard and Bradshaw 1964, Zobel and Talbert 1986). Genotypes that show least sensitivity or greatest buffering to environmental changes are selected and propagated. The magnitude of sensitivity of genotypes to environmental variations is generally estimated using phenotypic stability models (Finlay and Wilkinson 1963, Eberhart and Russell 1966, Hanson 1970, Freeman 1973). These models assume that the most stable genotype will show the least variance for a given trait across environments. With this assumption, stability is measured in terms of regression (stability index or "b" value) of performance of a genotype in different environments on the respective means of all genotypes. The most stable genotype is characterized by a regression coefficient of 1, with minimum deviation from regression.

In practice, genotypes are grown under different environmental (field) conditions; the "b" values are subsequently estimated for the character of

---

[1]Research Associate, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[2]Professor of Forest Genetics, University of Alberta, Edmonton, Alberta, Canada.

The authors thank S. Friedman and D. B. Wagner for comments, and NSERCC (F-0041) for financial support to D.R.G.

interest. Also, the stability values are generally estimated at a single developmental stage in the life history of the organism. In addition, evaluation of large number of genotypes under a wide array of environmental conditions is often a formidable task, particularly in tree breeding programs. Therefore, if a positive significant correlation between stability indices at different developmental or demographic stages or both, consistently exist, for similar set(s) of environmental conditions, then it is possible to select genotypes under laboratory (greenhouse) conditions. The objective of this study was to examine the relationship between stability indices estimated on a number of genotypes of jack pine (Pinus banksiana Lamb.), under similar sets of environments, at two different developmental stages. Germinating seeds were used to estimate phenotypic stability for the following reasons: a) the rate of germination is a genetically controlled trait (Whittington 1973), and thus influences the subsequent development of growth of plants (Hayes et al. 1955, Harper 1977), and b) a large number of families could be evaluated economically under extremely diverse environmental conditions in a very short time.

METHODS

The seed material used in this study consisted of 40 open-pollinated families from a single stand in Central Alberta. Two sets of experiments were carried out simultaneously using the same seed material. The experiments were laid out in a randomized block design with four replications. Experiment 1 consisted of four arbitrary levels of salt (water) stresses; they are: 1) control (deionized water), and sodium chloride, 2) -1.14, 3) -2.29, 4) -3.43 bars. Ten seeds from each family were placed in 9 X 1 cm petri dishes on Whatman No. 1 filter paper, and each petri dish was considered as a replication. Filter papers in the petridishes were moistened once every three days with 5 ml of the appropriate solution from the day of sowing. The seeds were allowed to germinate for 12 days under greenhouse conditions. Data were recorded on five randomly chosen radicles from each family and each replication. Experiment 2 was initiated with fifty seeds from each of the forty families. These were germinated separately, on a thin layer of inert sand, in a mist chamber. Ten, 10-day old seedlings were selected at random from the fifty seeds of each family, and were transplanted at the rate of one seedling per pot in each of the four media. The media included sand (medium 1; pH = 8.6), silt (medium 2; pH = 6.8), 50:50 peat-vermiculite mixture (medium 3; pH = 4.5), and peat (medium 4; pH = 3.8), placed in 2.5 X 2.5 X 5 cm seedling trays. The seedlings were grown under greenhouse conditions for 120 days. At the end of the experiment, all ten seedlings from each of the families and treatments were harvested at the root collar level, and their fresh weight immediately determined. Data on mean radicle length and biomass of each family from the each environment were used to calculte stability indices according to Eberhart and Russell (1965). The relationships between mean radicle length, biomass production and their respective phenotypic stabilities, and between the phenotypic stabilities of radicle length and biomass were calculated using Pearson correlation coefficients.

Table 1.--Mean radicle length, biomass production and phenotypic stability values of various families under two sets of environment.

	Radicle length (cm) under salt stress					Biomass (g) in soil media				
Family	Control	-1.14 bars	-2.29 bars	-3.43 bars	"b" values	Sand	Silt	Peat-verm.	Peat	"b" values
1	2.740	0.913	0.333	0.100	1.092	0.258	0.681	2.841	3.051	0.925
2	2.713	1.080	0.386	0.100	0.839	0.506	0.676	3.242	3.400	1.091
3	3.240	1.720	0.686	0.173	0.822	0.483	0.351	2.596	3.018	0.950
4	2.660	1.173	0.313	0.100	0.939	0.504	0.420	2.833	3.014	0.976
5	3.613	1.520	0.500	0.106	0.812	0.397	0.840	3.080	4.050	1.200
6	3.630	0.946	0.213	0.100	1.099	0.176	0.302	2.563	2.240	0.856
7	3.773	1.530	0.680	0.153	1.149	0.471	0.574	3.392	3.018	1.061
8	2.773	1.186	0.533	0.100	1.120	0.651	0.550	3.726	3.937	1.280
9	3.430	1.560	0.586	0.100	0.821	0.540	0.705	3.835	4.581	1.440
10	4.040	1.920	0.906	0.226	1.031	0.344	0.647	3.106	3.491	1.128
11	3.426	0.900	0.333	0.100	1.165	0.376	0.452	2.870	3.057	1.017
12	4.433	1.106	0.120	0.100	1.067	0.241	0.564	2.737	2.438	0.872
13	5.093	1.120	0.193	0.100	1.425	0.440	0.420	3.032	3.505	1.133
14	1.913	0.706	0.320	0.100	1.634	0.360	0.720	3.713	3.211	1.163
15	2.680	0.986	0.406	0.100	0.567	0.420	0.600	4.066	4.006	1.403
16	2.273	0.740	0.573	0.126	0.808	0.400	0.756	4.260	4.926	1.616
17	3.473	1.620	0.840	0.133	0.648	0.292	0.534	3.790	3.680	1.324
18	4.373	1.633	0.800	0.120	1.003	0.264	0.510	4.000	4.622	1.575
19	3.920	1.100	0.313	0.133	1.308	0.572	0.910	4.200	3.822	1.302
20	5.040	1.473	0.386	0.100	1.225	0.184	0.400	2.880	3.034	1.067
21	4.613	1.646	0.680	0.120	1.591	0.400	1.062	3.242	4.038	1.189
22	2.200	0.766	0.220	0.100	1.404	0.362	0.440	3.498	3.272	1.182

(continued)

Table 1. Continued.

	Radicle length (cm) under salt stress					Biomass (g) in soil media				
Family	Control	-1.14 bars	-2.29 bars	-3.43 bars	"b" values	Sand	Silt	Peat-verm.	Peat	"b" values
23	2.913	1.193	0.406	0.133	0.676	0.302	1.042	3.054	3.904	1.153
24	4.026	2.073	0.893	0.253	0.877	0.360	0.542	4.544	4.212	1.558
25	6.010	1.570	0.900	0.353	1.158	0.356	0.338	3.254	2.474	0.984
26	4.220	2.140	0.826	0.420	1.790	0.192	0.426	3.090	2.666	1.020
27	4.920	2.220	1.480	0.560	1.188	0.192	0.484	3.000	3.400	1.152
28	3.860	1.073	0.640	0.386	1.315	0.242	0.678	4.524	3.616	1.431
29	2.866	1.420	1.190	0.580	1.113	0.744	0.580	2.850	4.600	1.239
30	4.186	1.786	0.806	0.533	0.669	0.306	0.532	2.932	2.762	0.968
31	4.153	1.280	0.486	0.106	1.164	0.404	0.512	3.656	2.950	1.120
32	3.326	1.093	0.280	0.100	1.282	0.176	0.344	3.694	2.950	1.208
33	4.773	1.626	0.486	0.100	1.040	0.350	0.792	4.600	4.154	1.517
34	4.273	1.360	0.320	0.100	1.487	0.260	0.494	3.200	2.374	0.950
35	2.866	0.950	0.246	0.100	1.345	0.240	0.578	3.680	3.130	1.190
36	3.653	1.100	0.466	0.193	0.889	0.234	0.686	3.150	3.730	1.207
37	4.990	1.300	0.400	0.100	1.103	0.250	0.796	3.120	4.430	1.330
38	1.460	1.180	0.320	0.106	1.565	0.426	0.832	4.512	4.084	1.462
39	3.053	1.546	0.453	0.173	0.414	0.248	0.496	3.374	3.274	1.177
40	2.600	0.946	0.413	0.120	0.909	0.352	0.628	3.458	3.688	1.236
Mean	3.605	1.330	0.533	0.175	1.088	0.356	0.597	3.436	3.478	1.191
S.D.	0.983	0.385	0.287	0.136	0.307	0.020	0.281	0.090	0.105	0.198

RESULTS AND DISCUSSION

The mean radicle length in the germination experiment was higher in the (deionized water treatment), as expected, than under any of the other three stresses; and decreased with the magnitude of stress. On the other hand, in the seedling experiment, biomass production was highest on the peat-vermiculite medium, but lowest in sand medium. The phenotypic stability values for radicle length and for biomass varied from 0.856 to 1.616, and 0.414 to 1.790 respectively (table 1). An insignificant but positive association was found between radicle length and its "b" values (figure 1a, $r = 0.038$). On the other hand, a significant positive association was found between biomass production and its phenotypic stability (figure 1b, $r = 0.937$) the correlation coefficient of "b" values between radicle length and biomass production was slightly negative and insignificant (figure 2, $r = -0.145$).

The data presented in Table 1 indicate the following: 1) not all families that showed a high degree of stability ($b \simeq 1$) for highly stable genotypes at germination stage show a similar response at seedling stages; 2) only two families (27, 31) which showed relatively high stability ($b = 1.120 - 1.188$) for radicle length also showed a similar trend for biomass production; 3) when the criterion of minimum deviation (ie. maximum stability, $b = 1$) was ignored, twenty percent of the families showed similar stability values (± 0.1) for radicle length and biomass production.

These results, although preliminary in nature, indicate that the average performance of a genotype across environments (or sets) and developmental stages is largely unpredictable. Although Mather (1953) suggested that average response of genotypes to environmental stresses may be genetically controlled, the relationship between stability and productivity is not universal (Hardwick 1981); individual families show either specific or general environmental sensitivities (Conolly and Jinks 1982). Accordingly, as found in this study, certain genotypes may show some degree of stability consistently across environments and also across developmental stages. However, a general relationship among stability indices across environments, across developmental and demographic stages is difficult to obtain. Recently, Huhn and Leon (1985) also reported inconsistent relationships between stability parameters and means of lines in different years on Brassica napus, and cautioned against making generalizations using stability parameters in plant breeding programs.

The inconsistent relationship between stability values at two developmental stages could be due to the fact that data on radicle length and biomass were obtained on different individuals of the same family raised under two sets of environments. It has been suggested that any quantitative character of a genotype when measured in different environments must be considered as different characters, because in each environment, genes associated with the expression of quantitative characters respond differently (Falconer 1954, Yamada 1962). A similar argument could be made for characters measured at different developmental and/or demographic stages. Indeed, age-specific gene action in the evolution of age-structured populations, such as forest trees, is well-known (Charlesworth 1980). Furthermore, a linear and a

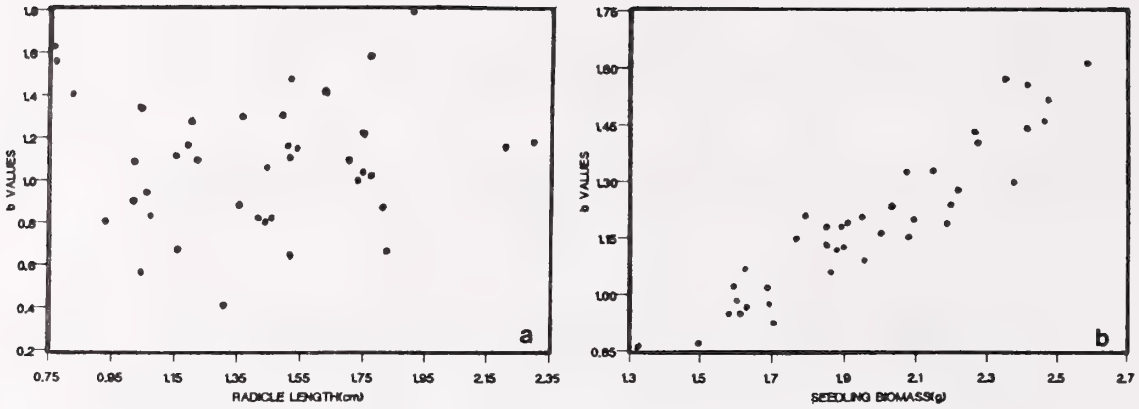


Figure 1. a. Association between mean radicle length of different families and and their 'b' values (r = 0.038; p = N.S.); b. association between mean seedling biomass of various families and their 'b' values (r = 0.937; p $\leq$ 0.0001).

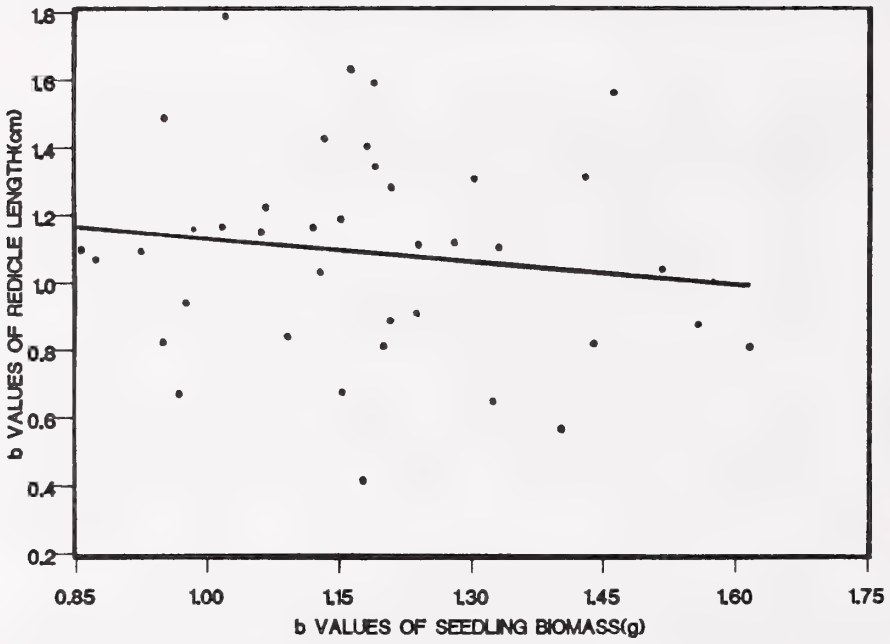


Figure 2. Association between the 'b' values of mean radicle length and mean seedling biomass (r =-0.145; p = N.S.).

tripartite relationship among phenotypic stability, heterosis and productivity has been reported for various crop plants (Adams and Shank 1959, Allard 1961, Pfahler 1966). These concepts (particularly phenotypic stability) have been considered as equivalent and commutative to the concept of homeostasis (Baker 1981). Nevertheless, reports indicate that phenotypic stability fluctuates over years and environments (Weber and Wricke 1986, Huhn and Leon 1985). Also the expression of both heterosis (Williams 1959, Griffing and Zsiros 1971, Barlow 1982, Govindaraju and Dancik 1987) and homeostasis (Orzack 1985) is strongly dependent on environmental variation. Therefore, we suggest that, although many important advances have been made in plant and forest tree breeding programs, using the concept of phenotypic stability its use as a universal predictor of stability across spatial, temporal, developmental and demographic differences based on correlated traits may be limited.

LITERATURE CITED

Adams, M. W. and D. B. Shank. 1959. The relationship of heterozygosity and homeostasis in maize hybrids. Genetics 44:777-786.

Allard, R. W. 1961. Relationship between genetic diversity and consistency of performance in different environments. Crop Sci. 1:127-133.

Allard, R. W. and A. D. Bradshaw. 1964. Implication of genotype X environment interaction in applied plant breeding. Crop Sci. 4:503-508.

Barlow, R. 1981. Experimental evidence for interaction between heterosis and environment in animals. Anim. Breed. Abst. 49:715-737.

Becker, H. C. 1981. Correlations among some statistical measures of phenotypic stability. Euphytica 30:835-840.

Charlesworth, B. 1980. Evolution in Age Structured Populations. Cambridge Univ. Press, Cambridge.

Connolly, V. and J. L. Jinks. 1975. The genetical architecture of general and specific environmental sensitivity. Heredity 35:249-259.

Eberhart, S. A. and W. A. Russell. 1966. Stability parameters for comparing varieties. Crop Sci. 6:26-40.

Falconer, D. S. 1952. The problem of environment and selection. Amer. Nat. 86:293-298.

Finlay, K. W. and G. N. Wilkinson. 1963. The analysis of adaptation in a plant breeding program. Aust. J. Agric. Res. 14:742-754.

Freeman, G. H. 1973. Statistical methods for the analysis of genotype-environment interactions. Heredity 31:339-354.

Govindaraju, D. R. and B. P. Dancik. 1987. Allozyme heterozygosity and homeostasis in germinating seeds of jack pine. Heredity (in press).

Griffing, B. and D. E. Zsiros. 1971. Heterosis associated with genotype environment interactions. Genetics 68:443-455.

Haldane, J. B. S. 1946. The interaction of nature and nurture. Ann. Eugen. 13:197-205.

Hanson, W. D. 1970. Genotypic stability. Theor. Appl. Genet. 40:226-231.

Hardwick, R. C. 1981. The analysis of genotype X environment interactions: what does it mean if varietal stability in linearly related to varietal performance? Euphytica 30:217-221.

Hayes, H. K., Immer, F. R. and D. C. Smith. 1955. Methods of Plant Breeding. McGraw-Hill, New York.

Huhn, M. and J. Leon. 1985. Genotype X environment interactions and phenotypic stability. Z. Pflanzenzuchtg. 95:135-146.

Mather, K. 1953. Genetical control of stability. Heredity 7:297-336.

Orzack, S. H. 1985. Population dynamics in variable environments V. The genetics of homeostasis revisited. Amer. Nat. 125:550-572.

Pfahler, P. L. 1966. Heterosis and homeostasis in rye (Secale cereale L.) II. Production of varieties, intravarietal crosses and composite population under various population densities. Crop Sci. 6:401-405.

Whittington, W. J. 1973. Genetic regulation of germination. In Heydecker, W. (ed.) Seed Ecology. The Pennsylvania State University Press, University Park.

Williams, W. 1959. Heterosis and the genetics of complex characters. Nature 184:527-530.

Wricke, G. and W. E. Weber. 1986. Quantitative Genetics and Selection in Plant Breeding. Walter de Gruyter, Berlin.

Yamada, Y. 1962. Genotype by environment interaction and genetic correlation of the same trait under different environment. Jap. J. Genet. 37: 498-509.

Zobel, B. J. and J. T. Talbert. 1984. Applied Forest Tree Improvement. John Wiley and Sons, New York.

FIELD AND GREENHOUSE FUSIFORM RUST SYMPTOMS PREDICT MORTALITY IN PROGENY FIELD TESTS

C. H. Walkinshaw[1/]

Abstract.--Six annual field observations on 43 slash pine families in a progeny test at Jackson County, Florida, showed that gall type was closely related to rust-associated mortality. In the field, the number of galls per family that developed from infection of terminal shoots was the single best predictor variable (r^2 = .85) for rust-associated mortality at 8 years. The proportion of trees with normal stems at age 5 or 6 years ranged from 25 to 100% and appeared to be a good predictor of mortality. Greenhouse inoculations of 21 of the 43 pine families in the progeny test showed purple stem spots without swelling to be the best variable for predicting field infection and mortality. Increasing the number of variables to three gave an r^2 of .64 for predicting field incidence. Differences in virulence of *Cronartium quercuum* f. sp. *fusiforme* collections in this progeny test were not related to disease severity among the pine families.

Additional keywords: *Pinus elliottii*, fusiform rust, disease severity, pathogenic variation.

Slash pines (*Pinus elliottii* Engelm. var. *elliottii*) that are infected by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* exhibit a variety of external symptoms in the field (Froelich et al. 1983). The effects of the rust include innocuous branch swellings, loss of apical dominance, girdling of the main stem, and mortality. Parent trees are considered susceptible to the rust when high numbers of progeny are galled, many cankers occur per tree, and when early infection and a fast rate of disease development occur. Resistant trees are more difficult to define. Both infection and symptom expression should be considered. In this study, the objective was to examine repeatedly a variety of symptoms in a typical progeny test. These symptoms were related to rust-associated mortality in the planting and to greenhouse symptoms for 21 of the 43 families. Also, virulence of the pathogen within the progeny test was measured as a possible variable in disease development.

[1/]Principal Plant Pathologist, USDA, Forest Service, Southern Forest Experiment Station, P. O. Box 2008, GMF, Gulfport, MS 39505.

METHODS

Definition of Terms

Innocuous branch galls occur on branches 30 cm. or more from the stem. *Branch-to-stem galls* are branch galls that are within 30 cm of the stem or which contact the stem, there is no swelling of stem tissues. *Branch galls in the stem* originate from a branch infection and are in the stem; stems are swollen. *Distorted stems* are trees bent or twisted from gall growth. *Normal stems* are straight trees with or without galls and with a single dominant terminal. *Rust-associated mortality* (RAM) is the number or proportion of trees that, in the opinion of the field observer, die from rust compared to the number of trees that survived planting. *True stem galls* are stem swellings that develop from the infected terminal shoot. *Virulence* is the property of the rust fungus isolate that permits it to attack one slash pine family more than another.

Progeny Test Observations

The Jackson County progeny test site, commonly called the "Bumpnose Site," is located near Greenwood, Florida (Goddard and Schmidt 1979). Commercial loblolly pines (*P. taeda* L.) are 95-97% galled in plantings adjacent to the test. Forty-three families of slash pine are planted in 5 replications of 10-tree row plots in this progeny test. Overall survival at age 3 was 62%. High mortality at planting occurred in the 4th and 5th replications. A total of 1,333 trees are being observed yearly for 18 different gall and tree characteristics beginning at age 3 in 1980.

Greenhouse Inoculation

Twenty-one (number based on seed availability) of the 43 slash pine families were inoculated with a standard 30-gall composite (S-6) at the Resistance Screening Center. Ten families had little resistance while 11 had potentially useful resistance. All procedures paralleled those of Laird and Phelps 1975 and Webb et al. 1984. The test had 2 runs of 3 trays of 20 seedlings each. After 6 months, 11 symptoms were read by the author. Resistant checks were included for comparison to former studies (Bey and Walkinshaw 1981, and Walkinshaw and Bey 1981).

Pathogen Variability Assessments

Aeciospores were collected from bright orange, sporulating stem galls on 30 trees. Two random diagonal passes were made for collection. Screened spores were shipped to the Resistance Screening Center to prepare inocula for measuring infection of Georgia bulk seed (susceptible check), 25-61 X W, 179-55 X W, and 18-55 X W (resistant check). These families possess the resistance necessary to measure the virulence of rust isolates (Bey and Walkinshaw 1981). Three runs of 3 trays of 10 seedlings each per family were inoculated. A 30-gall composite made from all the collections, 12 single gall isolates randomly chosen from the 30 collections, and the composite from the 21-family test were the inocula. After 6 months, observations were made by the author.

RESULTS

Progeny Test Observations

Proportion of trees with *branch galls in the stem* at 5 or 6 years was unrelated to RAM at 8 years (Fig. 1). Adding *proportion innocuous branch galls* and *branch-to-stem galls* did not improve the prediction for the 43 families. Proportion of galled trees had an r^2 of 39% with RAM at 8 years. Number of *true stem galls* at age 5 was a good predictor of rust mortality at age 8 (Fig. 2). *Normal stems* and RAM at 8 years had an r^2 of 65% (Fig. 3). Percent true stem galls and percent normal stems at age 5 had an r = -0.79.

Gall length, size of swelling, constriction or sunken areas, and a variety of other tree traits were poor predictors of RAM at 7, 8, or 9 years. Rust-associated mortality at 9 years is given in Table 1. Further mortality is projected on the assumption that existing infected trees may eventually die from rust. Note the good performance of the "C" families. See Goddard et al. 1975, for the basis of these selections.

Families in Table 1 with 1987 RAM values of 50% or higher had a mean of 0.22 for proportion of galled seedlings with witches' broom symptom. Those with lower RAM values had a mean of 0.15. Families C-71 and 25-61 had no witches' broom trees. The projected mortality of 63% for family C-71, which had the lowest RAM 1987, was due to a high incidence of stem galls formed from branch infections. Disease severity in family C-115 was initially more severe than in family C-71. By 1987, 38% of the trees in C-115 had died of rust, but only 3 trees remained with galls in the stems. This pattern was seen in a number of families as evident from present and possible RAM values.

Greenhouse Inoculations

Mean percent galled trees was similar across 21 5-year-old pine families in the progeny test and 6-month-old seedlings at the Resistance Screening Center (Table 2). Ten pine families had more rust infection in the field than at the Center. Proportion with symptoms but no swellings (SYMNO), fat galls, and smooth galls in the greenhouse predicted the Florida rust score with an r^2 of 64 percent (see Walkinshaw et al. 1980 for terms). The best single greenhouse predictor variable for field infection at age 5 and field RAM at age 9 was SYMNO with r-values of -.79 and -.64, respectively.

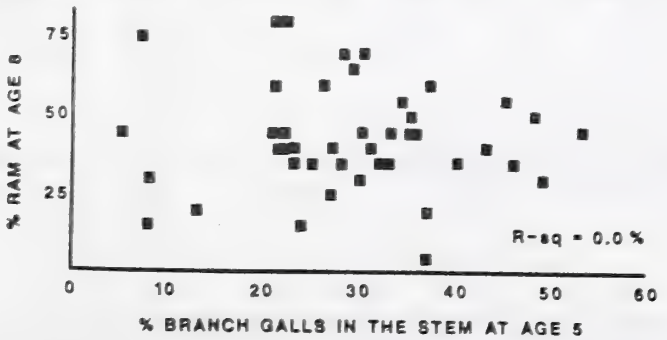


Figure 1. Lack of a relationship between stem galls from branch infections and early mortality.

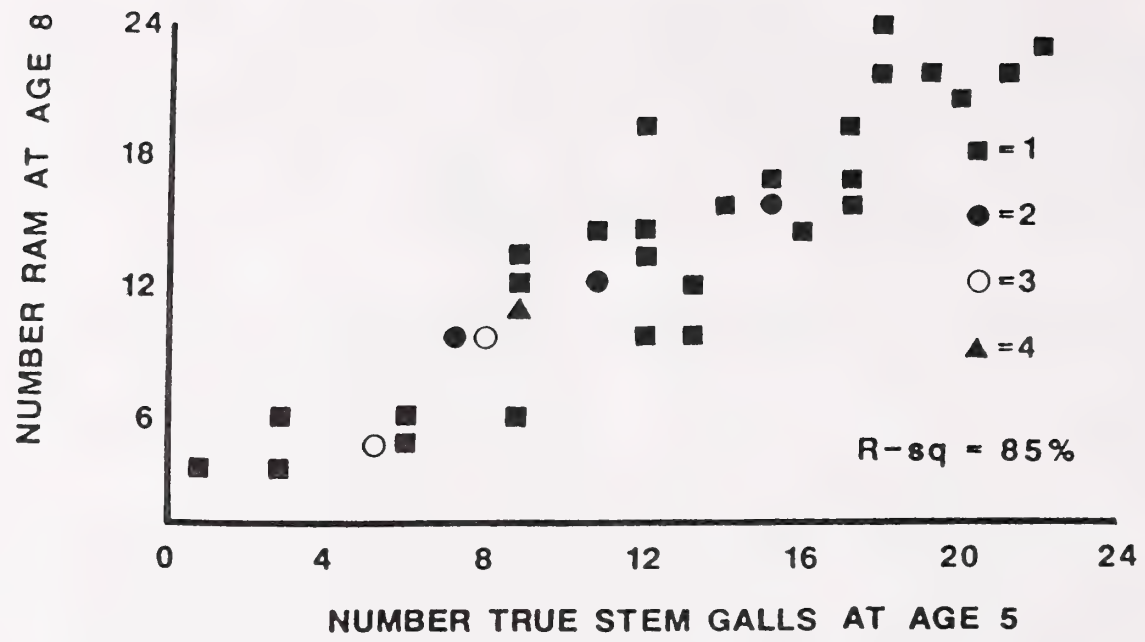


Figure 2. Relationship between early mortality and galls formed from infection of the terminal shoot.

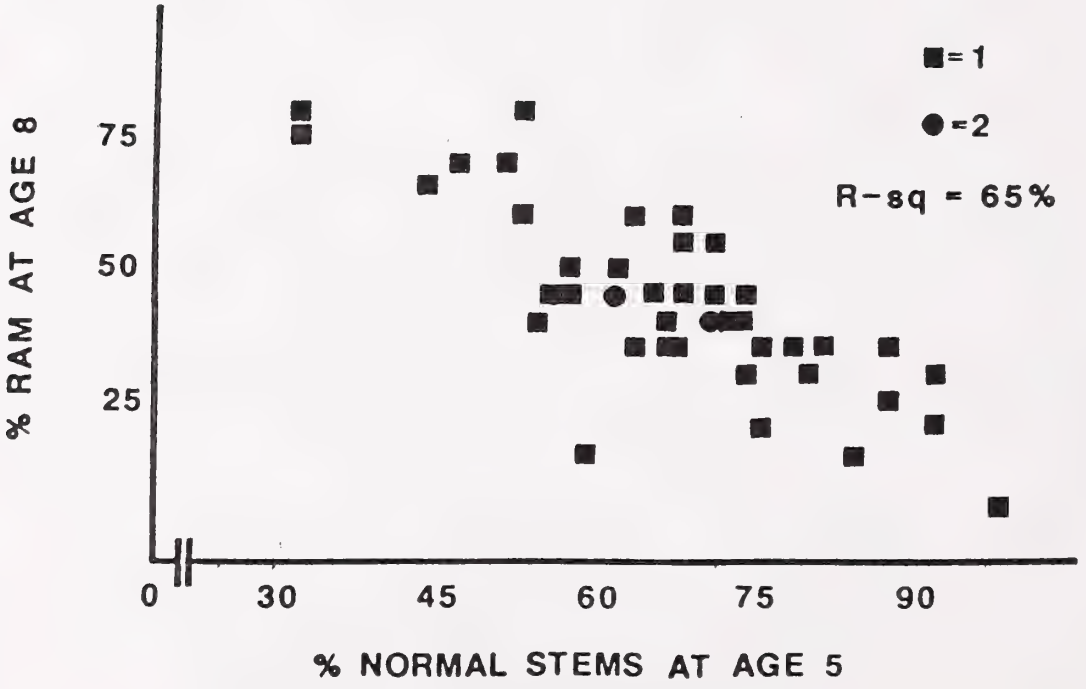


Figure 3. Incidence of normal stems (with and without galls and a single dominant stem) in relation to rust mortality at 8 years.

Table 1.--Existing and projected percent rust associated mortality (RAM) in slash pine planted near Greenwood, Florida

Family	1987 RAM	Possible RAM[1]
M-305	50	97
C-213	27	54
C-184	20	28
262-55	79	84
317-56	68	94
282-55	68	90
C-108	44	68
25-61	16	34
121-56	84	100
M-601	39	58
14-57	48	82
271-56	67	89
103-60	48	90
32-59	29	68
Check	30	65
26-61	55	84
238-56	60	88
B-1008	42	67
100-57	46	75
41-61	57	91
189-57	47	60
C-115	38	54
C-163	37	73
49-57	28	39
58-60	50	100
91-58	60	93
261-55	44	70
41-62	23	31
M-204	50	81
C-200	37	49
27-59	41	64
M-817	26	58
26-58	41	74
C-71	15	63
308-56	68	86
40-61	46	75
B-209	39	69
C-201	25	38
69-56	54	97
M-731	30	82
44-59	63	63
30-62	81	100
8-63	48	67

[1]Includes 1987 RAM (at 9 years) tree plus living trees with stem swellings.

Pathogen Variability Assessments

Infectivity of field isolates was unrelated to the resistance of the source family (Table 3). Differences among inocula were not significant at the 0.01 level. Family x inocula interaction was significant. Inocula separated resistant from susceptible families. Composite SC-30B appeared weaker on susceptible and resistant checks. Percent galled values for the single gall isolates on the resistant check (range 25 to 50%) were within previous values for 6 experiments with rust isolates (Bey and Walkinshaw 1981). Pine family 25-61 had a mean infection of 39% for all inocula, while the percent galled in the progeny test was 40. The highest percent galled for family 25-61 in the greenhouse occurred with an isolate from the most susceptible family. The isolate with the highest infection on family 179-55 also came from a highly susceptible pine family.

DISCUSSION

Results in this paper pertain to a single location and a limited period of tree growth. Studying only one location could certainly affect conclusions, although family X location interactions have not been a serious problem for these tests (Goddard and Schmidt 1979). Nine years appear sufficient to measure accurately early mortality from true stem galls when initial stem infection occurs at 1-3 years. More time is needed to define the effect of branch galls grown into the stem. Possible RAM values in Table 1 appear useful to compare rust among slash pine families. Rating families

Table 2.--Comparison of percent galled slash pine seedlings after 5 years in the field or 6 months after inoculation in the greenhouse.

Pine family	Florida planting[1]	Greenhouse[2]
C-71	32	21
C-200	45	45
C-213	45	47
41-62	12	35
M-601	57	40
C-115	35	46
189-57	46	54
32-59	65	53
B-209	56	62
317-56	82	73
M-731	66	72
261-55	47	47
26-58	65	63
308-56	72	53
69-56	85	67
271-56	32	73
41-61	80	74
M-305	70	73
14-57	68	27
58-60	67	77
121-56	60	77
Mean	56	56

[1]Mean of 4 or 5 replications of 10-tree plots.

[2]Mean of 3 replications with 3 trays of 20 seedlings each.

for incidence of true stem galls at age 5 would be of limited value for judging families such as 32-59, C-163, and C-71. This rating system would be more useful applied to resistant families C-184 and 49-57, and especially for susceptible families 262-55 and 121-56.

Data accumulated in this study support the contention that careful classification and early evaluation of gall category are required to evaluate mortality from stem galls (see Lloyd 1982, Nance et al. 1983, and Webb and Patterson 1983). The age of the tree at infection is certainly a critical variable (Froelich et al. 1983).

Comparing greenhouse and field infection of pine families indicates the disease severity at the Bumpnose Site. Generally, greenhouse infection is much higher than field, but this progeny test had a similar disease incidence to that in the greenhouse. Such a consistent level of fusiform rust infection strengthens conclusions made about these 1,333 trees.

The potential problem of rust pathogen variability (Bey and Walkinshaw 1981, Snow et al. 1976, van Buijtenen 1982, and Walkinshaw and Bey 1981) is partially addressed by data in this paper. Bey and Walkinshaw (1981) concluded that the buildup of virulent isolates should not be a serious problem. If the collections in this study were representative of the local population, then resistant families such as pines 179-55 and 25-61 would probably do well on the Bumpnose Site. Rust isolates from the Bumpnose Site did not appear as virulent as others we had collected from commercial plantings in Florida and Louisiana (e.g., FL-4 and LA-7 in Walkinshaw and Bey 1981). Fungus specialization did not seem abnormal in this progeny test. Moreover, incidence of one or more isolates of the fungus that were virulent on the best resistant pine families in this test were not indicated.

Table 3.--Infection of slash pine seedlings inoculated with field isolates of *Cronartium quercuum* f. sp. *fusiforme*[1]

Relative Resistance of aeciospore source[2]	Susceptible check	Resistant check	Family 25-61	Family 179-55
	% galled			
1.05	83	45	45	37
1.05	73	28	35	32
1.05	68	50	33	32
0.89	62	25	27	13
0.80	78	32	37	37
0.43	55	33	52	20
0.43	70	38	32	15
0.14	72	37	40	23
-0.24	77	42	37	52
-0.37	73	32	63	23
-0.37	72	50	38	25
-0.37	68	38	27	27
SC-30A	80	48	48	28
SC-30B	52	22	35	22

[1]Mean values for 3 replications of 10 seedlings each. Observations were made 6 months after inoculation.

[2]Evaluation from 1986 Florida data as defined in Walkinshaw et al. 1980. Spore source trees were selected at random from sporulating galls in the 5 replications. SC-30A is a composite of 30 galls in the planting; SC-30B is a composite of 30 galls in 5 counties to the northeast of the planting.

CONCLUSIONS

The numbers of stem galls that arise from infection of the terminal shoot appear to be the best field trait to predict rust-associated mortality. This evaluation should be made before branch-to-stem galls cause stem swelling. Combining incidence of true stem galls and stem galls from branches is not warranted for this planting. The proportion of trees with normal stems (straight trees with or without galls) at age 5 is one easy way to approximate mortality at age eight. This proportion is closely related to true stem gall. The high mortality observed in this planting appears to result from an abrupt growth retardation due to true stem galls. The decline is most evident in the sixth and seventh growing seasons when competition is beginning.

Disease incidence in this progeny test parallels incidence in greenhouse inoculations. Such a high infection in the progeny test indicates that conditions are ideal for the fungus at this location. This probably accounts for the strong negative correlation of SYMNO (resistance) in the greenhouse with field infection. Pathogenic variability does not appear to be an important variable in disease development.

LITERATURE CITED

Bey, C. F., and C. H. Walkinshaw. 1981. Stability of field resistant slash pine to selected isolates of fusiform rust fungus. Pages 107-114 *in* Proc. 16th South. Forest Tree Improv. Conf.

Froelich, R. C., W. L. Nance, and G. A. Snow. 1983. Size and growth of planted slash pines infected with fusiform rust. Forest Sci 29:527-534.

Goddard, R. E., and R. A. Schmidt. 1979. Relative geographic stability of resistance to fusiform rust of selected slash pine families. Pages 99-107 *in* Proc. 15th South. Forest Tree Improv. Conf.

Goddard, R. E., R. A. Schmidt, and F. Vande Linde. 1975. Effect of differential selection pressure on fusiform rust resistance in phenotypic selections of slash pine. Phytopathology 65:336-338.

Laird, P. P., and W. R. Phelps. 1975. A rapid method for mass screening of loblolly and slash pine seedlings for resistance to fusiform rust. Plant Dis. Reptr. 59:238-242.

Lloyd, F. T. 1982. Computer simulated fusiform rust losses from early infections in loblolly plantations. Pages 198-204 *in* Proc. Symp. Loblolly Pine Ecosystem (East Region).

Nance, W. L., R. C. Froelich, T. R. Dell, and E. Shoulders. 1983. A growth and yield model for unthinned slash pine plantations infected with fusiform rust. Pages 275-282 *in* Proc. 2nd Biennial South. Silvic. Res. Conf.

Snow, G. A., R. J. Dinus, and C. H. Walkinshaw. 1976. Increase in virulence of *Cronartium fusiforme* on resistant slash pine. Phytopathology 66:511-513.

van Buijtenen, J. P. 1982. A population genetic model of the alternate host system of *Cronartium quercuum* f. sp. *fusiforme*. Forest Sci. 28:745-752.

Walkinshaw, C. H., and C. F. Bey. 1981. Reaction of field-resistant slash pines to selected isolates of *Cronartium quercuum* f. sp. *fusiforme*. Phytopathology 71:1090-1092.

Walkinshaw, C. H., T. R. Dell, and S. D. Hubbard. 1980. Predicting field performance of slash pine families from inoculated greenhouse seedlings. USDA, Forest Serv. Res. Pap. SO-160. 6 p.

Webb, R. S., R. L. Anderson, and K. M. Portier. 1984. Comparative seedling resistance of *Pinus elliottii* var. *elliottii* and *P. elliottii* var. *densa* to *Cronartium quercuum* f. sp. *fusiforme*. Plant Dis. 68:145-148.

Webb, R. S., and H. D. Patterson. 1983. Effect of stem location of fusiform rust symptoms on volume yields of loblolly and slash pine sawtimber. Phytopathology 74:980-983.

APA: A USEFUL TOOL FOR ANALYSES OF PROGENY TESTS

S. B. Land, Jr.[1] and W. L. Nance[2]

Abstract.--Area Potentially Available (APA), an index of a tree's growing space, is a useful tool for adjusting family differences in basal area growth caused by differences in local density. APA also has utility in assessing family responses to changes in local density and to interfamily competition. Two loblolly pine progeny tests are analyzed to illustrate procedures, and the results have implications for testing and deployment of genetically improved varieties.

Additional keywords: Pinus taeda, stand density, interfamily competition.

All forest tree progeny tests are variable density studies. Each tree eventually has its own unique amount of growing space, or local density, as a result of (1) mortality of adjacent trees, (2) differential growth of the subject tree and adjacent trees, and/or (3) designed differences in spacing for stand density tests. Variable densities can lead to both problems and opportunities in the analyses of these progeny tests for family differences, once competition begins among adjacent trees.

Area Potentially Available (APA) is an index of local density for individual trees and has been described by Smith (1987) at this conference. Daniels et al. (1986) have shown that it is an excellent predictor of future growth of individual trees in closed stands. The present paper illustrates the application of APA in analyses of progeny tests to (1) adjust for differences in local density and (2) evaluate genetic differences in response to competition. Data from two loblolly pine (Pinus taeda L.) studies will be used to provide results for discussion.

MATERIALS AND METHODS

The APA Method

The APA index is the area of an irregular polygon constructed around a subject tree (Nance et al. 1983). The polygon is formed by intersecting lines (influence lines) that are located between and perpendicular to the lines connecting the subject tree with each of its competitors. The distance (LP) from the subject tree to the competitor's line of influence may be unweighted (half the distance between the two trees) or weighted by

---

[1]Professor, Department of Forestry, Mississippi State University, Mississippi State, MS 39762. Contribution No. 6697 of the Mississippi Agricultural and Forestry Experiment Station.

[2]Principal Plant Geneticist, Southern Forest Experiment Station, P. O. Box 2008 GMF, Gulfport, MS 39503.

the relative sizes of the two trees. In this paper the squared d.b.h. of each tree is used to weight the LP.

Progeny Tests

Data are used from two loblolly pine studies planted at the same site in northeast Mississippi (Oktibbeha County, 33°18'N latitude, 88°47'W longitude). Open-pollinated families from eight clones in the Weyerhaeuser Company seed orchard near Aliceville, Alabama, are represented in both studies. Origins of these clones are Lamar, Pickens, and Greene Counties in west central Alabama.

The first study, hereafter called the "Nelder's Study", has the families planted along spokes of a Nelder's Wheel design (Namkoong 1965). There are five spokes per family in a 42-spoke wheel (including two border spokes) that represents a replication, and there are ten replications. The five-spoke family plot is arranged in the spoke order -- A4-B1-A5-B2-B3-B4-C1-B5-C2 -- (where A, B, and C are different families). Spoke 3 of each family represents a "pure" single-family deployment, whereas the other four spokes are in "mixed" family deployments. There are five measurement positions and an interior and exterior border on each spoke, with the measurement positions having local densities of 1210 (6 x 6 feet), 938 (6.8 x 6.8 feet), 727 (7.7 x 7.7 feet), 563 (8.8 x 8.8 feet), and 436 (10 x 10 feet) trees per acre. Measurements of d.b.h. have been taken annually from age five to age ten.

The second study, hereafter called the "Design Study", contains three progeny tests having common families but different plot designs. The "Block Plots Test" has 40 trees per family planted in a 4 x 10-tree block plot in each replication. The "Row Plots Test" has 40 trees per family planted in four 10-tree row plots assigned randomly to 32 row positions in each replication. The "Non-Contiguous Plots Test" has 40 trees per family assigned randomly to 320 single-tree positions in each replication. All trees are planted at 8 feet x 8 feet (681 trees/acre), and there are six replications. Measurements of d.b.h. have been taken at ages 5, 7, 9, and 11 years after planting.

PROCEDURES AND RESULTS

A recommended stepwise procedure is given below for using APA in progeny test analyses to adjust for differences in local density and to evaluate genetic differences in response to competition. It should only be used after crown closure, when available growing space becomes limiting. Accomplishment of each step with SAS computer software (SAS Institute 1985) is described and illustrated with results for the two loblolly pine studies.

Step 1--Determine if differences in local density exist in the study and affect growth. A new computer program to calculate polygons and APA's may be obtained from W. L. Nance. The output file from the program contains the APA value and coordinates for the polygon of each tree. The coordinates can be used by SAS GRAPH to print a polygon map of the study for visual verification of results (Figure 1). The APA values can be merged with a SAS data

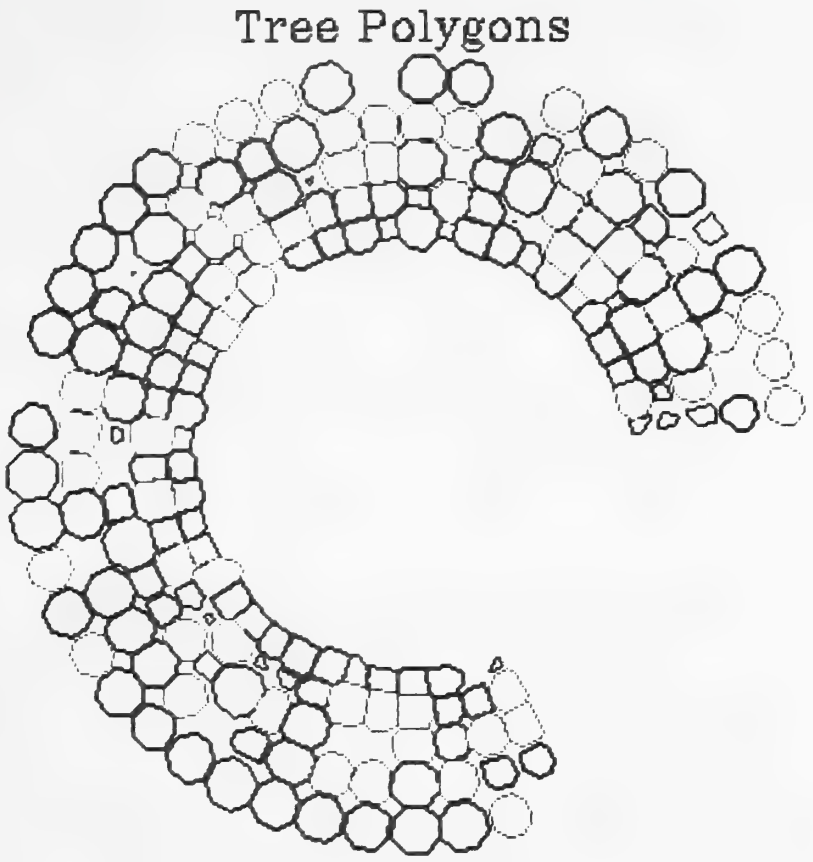


Figure 1. Polygon map of one replication of the Nelder's Study at age eight. Border trees are not shown in this illustration.

file containing basal area growth (BAG) for each tree, and the SAS procedure PROC REG can then be used to calculate the regression equation for BAG per tree from time "A" to time "B" as a function of the APA of the tree at time "A". Significance of regression and size of the coefficient of determination (R^2) will indicate whether or not local density differences are affecting growth. Local density differences existed in the loblolly studies after crown closure and explained from one-third to two-thirds of the variation in BAG, as indicated by the sizes of R^2 (Table 1).

Step 2--Determine if families are growing at different local densities. Family means and an analysis of variance for individual tree APA's can be obtained by the SAS procedures PROC MEANS and PROC GLM on the merged data file. If the "Family" source of variation for APA is significant, then families are growing under different local densities. Analyses of family differences for BAG in the following years will be biased by the effects of these differences in local density.

The Nelder's Study and the Non-Contiguous Plots Test had significant family differences in APA (Table 2). In both of these tests family 530 is growing under a significantly higher APA (lower local density) than family 509. Subsequent BAG should be greater for 530 than for 509 as a result of

Table 1. Regression equations for Basal Area Growth (BAG) per tree as a function of APA per tree in the Design Study and Nelder's Study.

BAG Interval start (Yr.)	BAG Interval end (Yr.)	Study Means BAG (in^2/tree)	Study Means APA at start (ft^2/tree)	Regression Equation	Adjusted R^2
			Design Study		
7	9	11.9	48.3	BAG = 2.1913 + .2043 APA	.39
9	11	9.8	61.2	BAG = 1.2141 + .1403 APA	.29
			Nelder's Study		
7	9	11.2	44.3	BAG = 1.6327 + .2122 APA	.62
8	10	10.6	50.9	BAG = 2.0541 + .1670 APA	.52

more space and resources being available to 530 for growth, but the results will not indicate how the two families would compare under equivalent competitional stress (same APA). That comparison may be very relevant for forest crops of the future, where high survival and homogeneity in tree sizes from intensive crop culture could result in less variability in APA's.

Table 2. Family mean APA's at age eight in the Nelder's Study and at age nine in the three progeny tests of the Design Study, with significance levels for F tests of family variation.

	Family Mean APA (ft^2/tree)			
		Design Study		
Family	Nelder's Study	Non-Contiguous Plot	Row Plot	Block Plot
505	51.2	61.3	61.2	59.1
507	51.3	62.4	59.2	58.8
509	47.7	57.4	58.2	63.2
519	51.0	59.0	59.8	58.2
526	50.4	59.9	60.8	57.8
528	51.3	61.6	59.3	59.7
530	52.6	63.5	61.5	60.8
532	51.1	61.6	58.0	61.5
Test Mean	50.8	60.9	59.8	59.8
Family F-test (PR F)	2.48 (0.025)	2.40 (0.041)	1.02 (0.436)	1.47 (0.210)

Step 3--Determine if families respond differently to changes in local density. The appropriate adjustment procedure for comparing families at the same local density will depend on whether or not families respond differently to changing APA. The SAS procedure PROC GLM can be used to test for homogeneity of slopes of the individual family regressions for BAG as a function of APA. The model contains two independent variables, APA and APA*FAMILY. A significant F-test of the Type I mean square for APA*FAMILY (coming after APA in the model) indicates that the slopes are not homogeneous. Should this happen, the separate family regression lines can be plotted to see if the lines cross and where the greatest family differences occur.

The APA*FAMILY interaction was significant in all of the loblolly tests. A plot of regression lines for the Non-Contiguous Plot Test revealed that families 509 and 530 were major contributors to this interaction (Figure 2). This is a G x E interaction that (1) creates problems for the

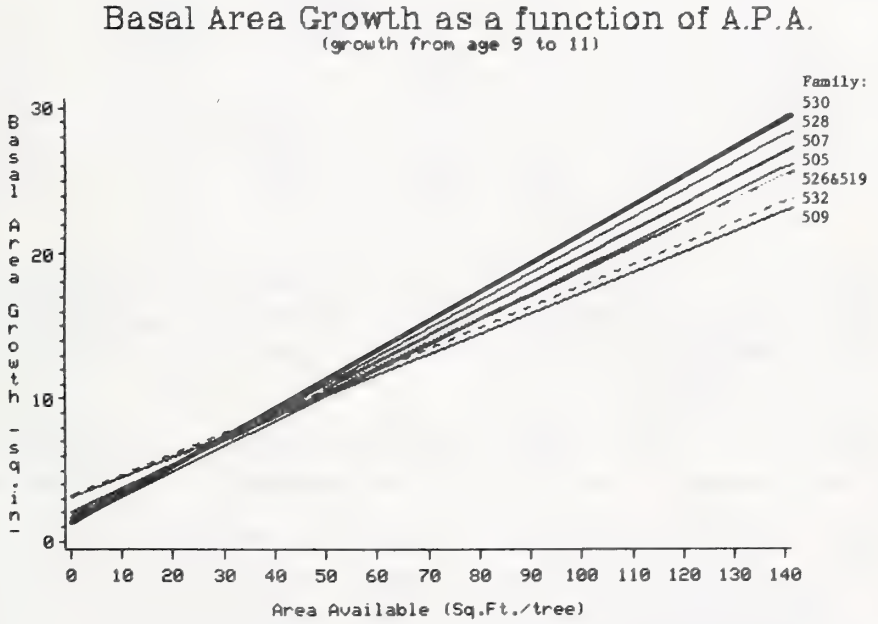


Figure 2. Separate family regression lines for Basal Area Growth per tree as a function of APA per tree in the Non-Contiguous Plot Test.

adjustment of families to comparable local densities (discussed below) and (2) raises questions about the need to conduct progeny tests over a range of densities (discussed later).

Step 4--Adjust for differences among families in local density. The reasons for adjusting individual-tree BAG values for differences in APA include (1) more accurate assessment of family differences for growth under competition and (2) reduction in error to provide greater precision in detecting family differences. The method of adjustment will depend, however, on

whether or not the APA*FAMILY interaction is significant. In those studies where no APA*FAMILY interaction exists (no slope differences), APA can be used as the covariate in a covariance analysis by PROC GLM to compare families adjusted to the study mean APA (left side of Figure 3). Family "a"

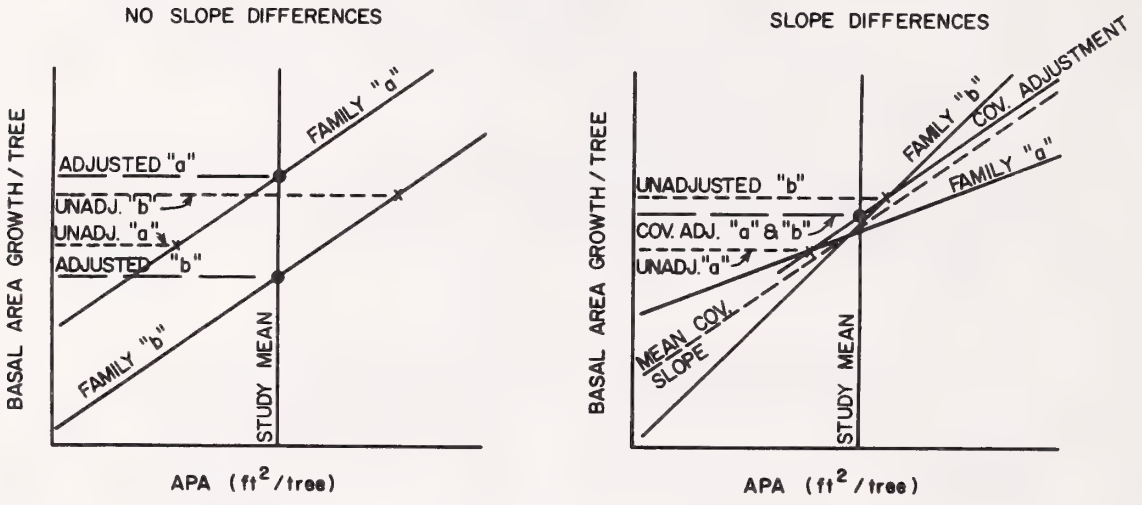


Figure 3. Schematic representation of using APA as a covariate to adjust family mean Basal Area Growth when no slope differences exist among family regressions (left) and when family regressions differ in slope (right).

that was growing at a lower APA than family "b" and exhibiting a lower measured BAG may actually be better than family "b" when adjusted to a common APA.

In studies where APA*FAMILY interactions are significant (slope differences exist), adjustment with the APA covariate will give only approximate tests of family differences near the study mean APA (right side of Figure 3). The covariance adjustment in that example results in no difference between families "a" and "b" at the study mean, but "a" is actually slightly poorer than "b" at that mean APA and much different at APA's distant from the mean. The use of APA and APA*FAMILY as multiple covariates, which is the same as using APA (FAMILY) as a single covariate, will adjust for both APA effects and family effects. Since we wish to study family effects, not remove them, this multiple covariance procedure is not appropriate. The authors recommend that when APA*FAMILY interactions exist, a target APA should be chosen and families compared for that target. This can be done by breaking the data file into separate APA classes, with a class centered on the target APA. PROC GLM can then be used to conduct an analysis of variance on the unbalanced data set for the trees in the target APA class.

Results from PROC GLM with Tukey's test of ranked family means for the Non-Contiguous Plot Test illustrate how simple covariance adjustment for APA reduced the error and family mean differences in BAG (Table 3). This is an

Table 3. Ranked family means for Basal Area Growth in the Non-Contiguous Plot Test before and after adjustments for differences in local density (APA).

Basal Area Growth (square inches per tree) from Age 9 to Age 11[a/]									
Not Adjusted for APA at Age 9		Adj. by APA Cov. to 60.9 ft^2 (1 slope)		File Separated into APA Classes					
				APA=15-45ft^2		APA=45-75ft^2		APA=75-105ft^2	
Family	Mean	Family	Mean	Family	Mean	Family	Mean	Family	Mean
530	10.8	530	10.4	519	6.7	530	10.4	528	14.7
528	10.3	528	10.2	528	6.3	507	9.9	530	13.8
507	10.0	526	9.8	509	6.1	526	9.8	507	13.2
526	9.7	507	9.7	530	5.8	532	9.8	509	13.1
505	9.5	519	9.6	526	5.7	528	9.7	519	12.4
532	9.5	505	9.5	532	5.5	505	9.6	532	12.3
519	9.4	532	9.4	507	5.4	519	9.5	505	12.3
509	8.7	509	9.2	505	5.0	509	8.8	526	11.9
MSE =	21.91		15.84		9.02		13.77		12.94
Tukey's Min. Sign. Diff. =	1.60		1.36		2.83		1.54		3.13

[a/]Means followed by the same continuous line are not significantly different at the 0.05 probability level.

approximate adjustment, since slopes for the individual family regressions were different. In fact, the only significant rank correlations (Steel and Torrie 1960) among the family means in the five columns of Table 3 are for (1) the unadjusted means versus the covariate-adjusted means and (2) the unadjusted means versus the means in the restricted APA class for 45-75 square feet. When APA*FAMILY interactions are significant, family superiority at one stand density level should not be extrapolated to other densities.

Step 5--Determine if families respond differently to the genetic composition of their neighboring competitors. The purpose of this step is to determine if family mean BAG is influenced not only by the amount of available growing space, but also by the genetic composition of the trees surrounding that growing space. An affirmative answer would imply that intergenotypic

competition exists among trees in these field studies, supporting results detected in seedling studies by Adams (1980) and Tuskan (1984).

Nance et al. (1983) have already described a procedure for using APA, APA*FAMILY of the subject tree, and the "relative influence" of each competitor family in a multiple regression model to predict subsequent BAG of the subject tree. The data were from the same Nelder's Study used here, but for BAG from age 7 to age 8. APA*FAMILY interactions (differences in subject family competitive abilities) contributed significantly to the predictive ability of the model, but relative influences of competitor families (intergenotypic interactions) were not significant.

Another approach for studying effects of intergenotypic competitive interactions is to compare family ranks in mixed-family deployments and in single-family "pure" blocks. Mixed-family deployments experience inter-family competition, while single-family blocks experience only intrafamily competition. If ranks differ in the two deployments, indicating the presence of interfamily competitive interactions, then plots of BAG over APA for the families can be used to help explain the interactions.

The Nelder's Study data were divided into (1) a "pure family" file containing only trees from spokes surrounded on both sides by spokes of the same family and (2) a "mixed family" file containing all other trees. The Block Plot Test provides a "pure-family" arrangement, the Non-Contiguous Plot Test provides a true "mixed-family" situation, and trees in the Row Plot Test experience a partial "mixed-family" and a partial "pure-family" situation. Family rank correlations for BAG (unadjusted for APA) were not significant between "mixed" and "pure" deployments within either the Design Study or the Nelder's Study, although the partial "mixed + pure" of the Row Plots correlated with both "mixed" and "pure" in the Design Study. However, significant correlations were obtained between the two studies for comparable deployments ("pure" versus "pure" and "mixed" versus "mixed"), indicating the repeatability of the interfamily competitive interactions.

Families 509 and 530 contribute greatly to the lack of a significant rank correlation between "mixed" and "pure" family deployments, with 509 being better in "pure" than in "mixed" situations of the Design Study and 530 being the opposite (Figure 4). The same relationship is found in the Nelder's Study. APA provides the mechanism for evaluating this relationship. The response of family 509 to changing local density is quite different in the "mixed family" competition of the Non-Contiguous Plot Test than in the "pure family" competition of the Block Plot Test, whereas family 530's response is quite similar in both situations (Figure 5). The key, however, is the response of 509 versus 530 in the mixed situation. When family 530 is planted in mixture with other families (such as 509), it grows faster than the other families under conditions of low competition (large APA) and captures growing space from the surrounding trees. In this sense family 530 is typical of the "competition ideotype" described for crops (Donald 1968). However, when 530 is grown in "pure" blocks it must compete with its aggressive siblings. Its mean APA per tree drops, and it performs no better across a wide range of APA's than family 509 competing with itself.

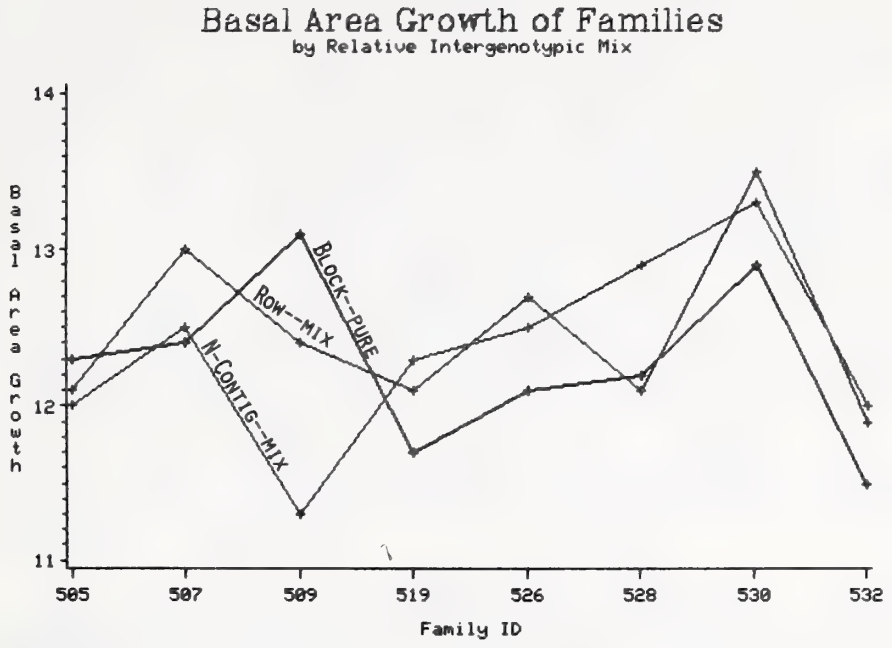


Figure 4. Family means for each of three progeny test designs having different family deployments in the Design Study.

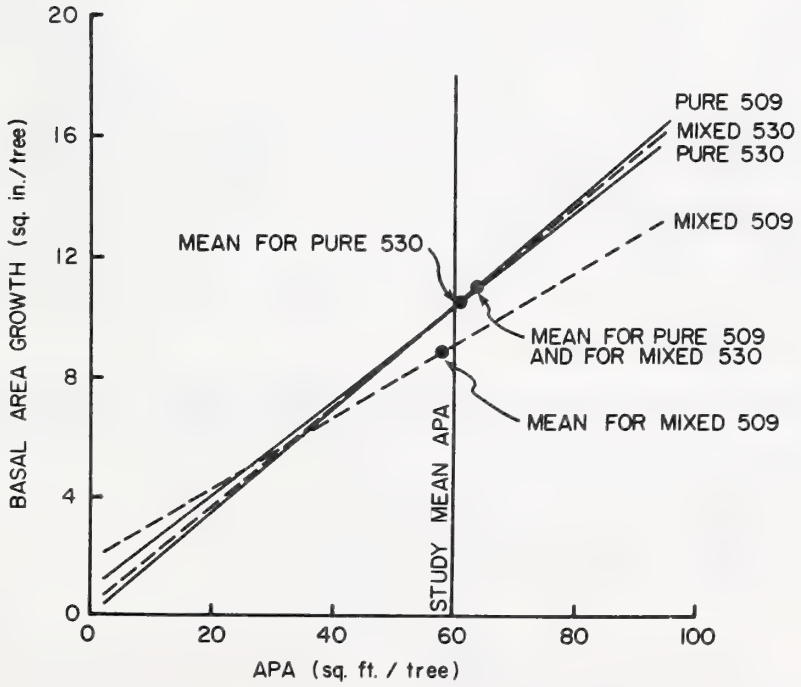


Figure 5. Family regression lines of Basal Area Growth from age 9 to 11 as a function of APA at age 9 for families 509 and 530 in the Non-Contiguous Plot Test (mixed) and in the Block Plot Test (pure).

Conversely, family 509 suffers from the competition of families like 530 when grown in mixture. It gives up growing space to trees from such families, and as a result its mean APA is lowered below the study mean APA. Its mean BAG is therefore low. Thus, when the family BAG means for 509 and 530 are compared in mixture, family 509 is much lower than 530 as a combined result of low APA and poor inter-family competitive ability (Figure 5). Even if the family means were adjusted to the study mean APA, family 509 would still be the loser as a result of its reaction to the genetic composition of the trees surrounding the growing space. Notice, however, that when family 509 is planted in a "pure" family stand it retains a higher mean APA per tree than the aggressive family 530 and has an equivalent growth response over the range of APA's. Since it has a greater mean APA than 530, its mean BAG per tree is greater in "pure" stands than is the mean for 530. If the family means were adjusted to the study mean APA, there would be no difference between the two families. Family 509 is illustrative of Donald's (1968) "crop ideotype", which maximizes performance in single variety plantings and does not do well in the heterogeneous competitive situations of mixed varieties or blends.

DISCUSSION AND CONCLUSIONS

APA can be used as a basis for adjustment for differences in local density. The method is not perfect. It should not be used as a simple covariate if family-by-APA interactions exist. Instead, the authors recommend subdividing the data into APA classes and examining family differences in growth within each class. Another complaint about the technique will be the fact that its calculation is dependent in part on the genetically-controlled trait "d.b.h.". Some of the genetic difference between families may be removed by the adjustment process. We are most interested here, however, in defining genetic differences in ability to utilize a limited growing space (growth efficiency when resources are limiting). This is different from genetic differences in ability to capture growing space from adjacent trees (prior aggressiveness). Both growth efficiency and aggressiveness contribute to a family's competitive ability, but the former may be more important for improving stand yields. Therefore, we are willing to accept the removal of some of the genetically-controlled aggressiveness in order to compare families for genetic differences in growth efficiency.

APA is useful for evaluation of genetic differences in response to competition. It can be used to study family response to changes in both the amount of growing space and the genetic composition of the surrounding trees. The significant family differences detected here in the loblolly pine examples have the following important implications for testing and deployment of genetically improved varieties.

First, families should be tested under a designed range of local densities. If not, errors may be made in selecting appropriate families for deployment at specific target densities. Second, families should be tested under a variety of competitive situations involving both pure block and family mixture plot designs. Otherwise, mistakes may be made in the family rankings for growth and in the families selected.

Some unanswered questions remain.

(1) Are there some families that perform best over a wide range of densities? Obviously, these would be the most desirable, because they would allow greater flexibility in deployment.

(2) If not, what type of family response is most desirable?

(a) Are families that rank best in BAG at high densities (low APA's) the most shade tolerant, least nutrient demanding, etc.? If so, should they be selected to allow maximization of stand yield by packing more trees per acre?

(b) Should one select for maximum growth per tree at low densities (high APA's), as this might allow the quickest achievement of merchantable size? Will such families require heavy and frequent thinning?

(3) Are there families that perform best in both mixed and pure family arrangements? Here again, these families would be most flexible in deployment strategies.

(4) If not, which is more desirable--crop ideotypes or competition ideotypes?

(a) Crop ideotypes are those families that perform best in pure family blocks at high densities. Are they the more desirable type for total fiber yield?

(b) Competition ideotypes are families that perform best in mixtures and/or low densities. Will they be best for rapid production of large-sized products?

APA is easy to use and can be applied to data already collected. Recent improvements in the APA computer program make it easily adaptable to any data set containing d.b.h. measurements and tree-position coordinates (usually row and column). It can be calculated for each living tree, which is desirable in progeny tests where analyses are based on individual-tree observations. Furthermore, it requires no special designs, so that it can be used immediately on existing progeny tests. Although refinements in calculations and the use of other measures of local density can and should be tested, APA is a useful tool for analyses of forest tree progeny tests today.

LITERATURE CITED

Adams, W. T. 1980. Intergenotypic competition in forest trees. Proc. Sixth North American Biology Workshop. The University of Alberta. p. 1-14.

Daniels, R. F., H. E. Burkhart, and T. R. Clason. 1986. A comparison of competition measures for predicting growth of loblolly pine trees. Canadian Jour. For. Res. 16:1230-1237.

Donald, C. M. 1968. The breeding of crop ideotypes. Euphytica 17:385-403.

Namkoong, Gene. 1965. Application of Nelder's designs in tree improvement research. Proc. Eighth Southern Conference on Forest Tree Improvement, Savannah, GA. Sponsored Publ. No. 24 of the Comm. on So. Forest Tree Impr. & Georgia Forest Research Council. p. 24-37.

Nance, W. L., S. B. Land, Jr., and R. F. Daniels. 1983. Concepts for analysis of genetic competition studies. Proc. Seventeenth Southern Forest Tree Improvement Conference. University of Georgia. p. 131-145.

SAS Institute. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute, Inc., Cary, NC. 956 pp.

Smith, W. R. 1987. Area potentially available: A research tool. Proc. Nineteenth Southern Forest Tree Improvement Conference. Texas A&M University. (In Press.)

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York. 481 pp.

Tuskan, G. A. 1984. An investigation of inter-family competition in loblolly pine. Ph.D. dissertation, Texas A&M University, College Station, TX. 97 p.

REGRESSION AND SPLINE METHODS FOR REMOVING ENVIRONMENTAL VARIANCE IN PROGENY TESTS

B. C. Bongarten and J. F. Dowd[1]

Abstract.--Regression and spline techniques were compared to blocking for their ability to model and eliminate environmental variation in progeny tests. With the regression and spline techniques, environmental gradients were determined by modeling trait response surfaces with respect to row and column position in the test. Deviations from the modeled surfaces were assumed to represent random genetic and environmental effects, and served as data for determination of variance components.

Fourth year height and diameter in a sycamore progeny test were used to demonstrate the method. Results show that the plot within family variance may be substantially reduced without affecting genetic variation using spline techniques. As a result, heritabilities were substantially higher than when blocking was used. Spline methods for eliminating environmental variation obviate the need for blocking altogether, and, thus, facilitate the establishment of progeny tests with large numbers of genetic entries. These methods will be most effective when single tree plots are employed.

Additional keywords: heritability, genetic selection.

The efficiency of progeny tests is largely dependent on the degree to which environmental variation can be separated from genetic variation. In progeny tests, environmental variation may be divided into two components: random variation (with respect to position in the progeny test), resulting from differences in microsite, stock condition, handling, etc., and non-random variation, resulting from environmental gradients in the site. In this work we address methods for eliminating the non-random sources of environmental variation.

This task is normally accomplished by blocking. However, blocking is inefficient because environmental gradients are not normally stepwise, but continuous. The erroneous assumption that environmental gradients are stepwise induces large family x block interactions because a family plot may occur on an "above-average" site in one block, and a "below-average" site in another block. More realistic modeling of environmental gradients should provide a means to reduce this source of variation and, thereby, improve heritabilities and the accuracy of among and within family selection.

---

[1] Associate and Assistant Professors, respectively, School of Forest Resources, The University of Georgia, Athens, GA 30602

Elimination of environmental trends in progeny test data may be accomplished by subtracting non-random environmental effects from the actual data. Non-random environmental effects may be estimated by fitting a smooth surface through the actual data, assuming that all genetic variation is random so that any trends are due solely to environmental causes.

Options for modeling trends include, among other things, polynomial regression and various spline techniques. Polynomials are simple, but they force the number of peaks and inflections and have limited flexibility. Therefore, they are unsuited to most situations. Splines, taken to their extreme, explain all variation. However, it is possible to fit splines to widely spaced "knots" using least-square methods. This technique is more flexible, but its effectiveness is dependent on determining the proper knot spacing. If knots are too close, some genetic variation will be eliminated; if they are too far removed, environmental trends will not be fully described.

Here, our objectives are to (1) demonstrate how these methods can reduce environmental variation, and therefore, increase heritability, (2) show how piece-wise spline knot spacing can affect genetic and environmental variances and (3) discuss how to apply these methods to maximum advantage.

MATERIALS AND METHODS

The example presented in this paper is from a sycamore (Platanus occidentalis L.) progeny test located in Putnam County, Georgia. The site is a level Piedmont bottomland. Before planting the site had been cleared of timber, stumped and cultivated. Subsoil variations have resulted in conspicuous gradients in site quality.

The progeny test was planted as a randomized complete block with 221 open-pollinated families, four blocks and two-tree plots. Tree spacing is 3 x 3 m. A double border row surrounds the perimeter. Heights and breast height diameters four years from planting were the data analyzed. Trees had just reached crown closure at the time of measurement.

The data were first analyzed using the planned ANOVAs for a randomized complete block design, and then using ANOVAs for a completely randomized design, after the environmental gradient effects estimated from the regression or spline models had been subtracted.

Models for estimating environmental gradient effects were constructed as follows. Curves were fit through the each row, independently, by least squares, using plot means. This procedure was then repeated for each column. For each plot, row and column estimates were averaged to produce a first approximation of the surface. The surface was smoothed by iteratively applying the row and column fitting procedure to the estimated values of the previous iteration. Less than ten iterations were necessary to provide a smooth approximation.

First, cubic polynomial regressions were used to model row and column curves. Then, piece-wise splines were used. In the piece-wise splines, piece lengths, hereafter called segments, were 6, 8, 10, 12 and 18 plots. This resulted in 5, 4, 3, 2 and 1 segment(s), respectively, for most rows and columns. Each segment was fit to a cubic polynomial by least-squares methods, subject to the constraints that the first and second derivatives of the curve segments must be the same at their unions. The resulting splines are, thus, referred to as least-squares piece-wise cubic splines.

Spline fitting was performed on a personal computer using programs adapted from deBoor (1978). These procedures will be detailed in a future paper. Cubic polynomial surfaces were fit with SAS (1982) procedure REG and with personal computer programs. Statistical analysis was conducted with SAS (1982) procedure VARCOMP.

For heritability calculations, block variation was excluded from the phenotypic variance term in the randomized complete block analysis, and the covariance among family members was assumed to be 0.25. Standard errors for heritabilities were calculated using the methods outlined by Namkoong (1979).

RESULTS

The results of environmental gradient modeling are portrayed by examining a slice through the progeny test along column Q (Figures 1-3). In Figure 1, curves resulting from the first approximation of the environmental gradient for height, determined by cubic polynomial regression and piece-wise splining with segment length of ten plots (spline 10), are shown. Highly irregular surfaces are produced from the first approximation. However, smooth surfaces are produced after ten iterations of surface fitting (Figure 2). From the smooth curves, it is evident that the piece-wise spline model was more "flexible" than the cubic polynomial model, producing peaks and valleys with greater amplitude and frequency. The effect of segment length on surface characteristics is shown in Figure 3. Spline surfaces constructed using short segment lengths have more flexibility than those using long segment lengths (e.g. compare the curve produced when segment length was six plots (spline 6) to that produced when segment length was 18 plots (spline 18)).

Greater flexibility means that more varition is explained by the the surface, leaving less for genetic and residual environmental variation. The effect of several environmental gradient models on family and residual environmental variance is shown in Figure 4. As expected, residual environmental variance was minimized when gradients were modeled using short segments in a piece-wise spline, and increased as the segments were lengthened (note that the polynomial regression is similar to a least-squares piece-wise spline where segment lengths equal the numbers of plots in each row or column). All of the smooth surface models resulted in less residual environmental variation than blocking.

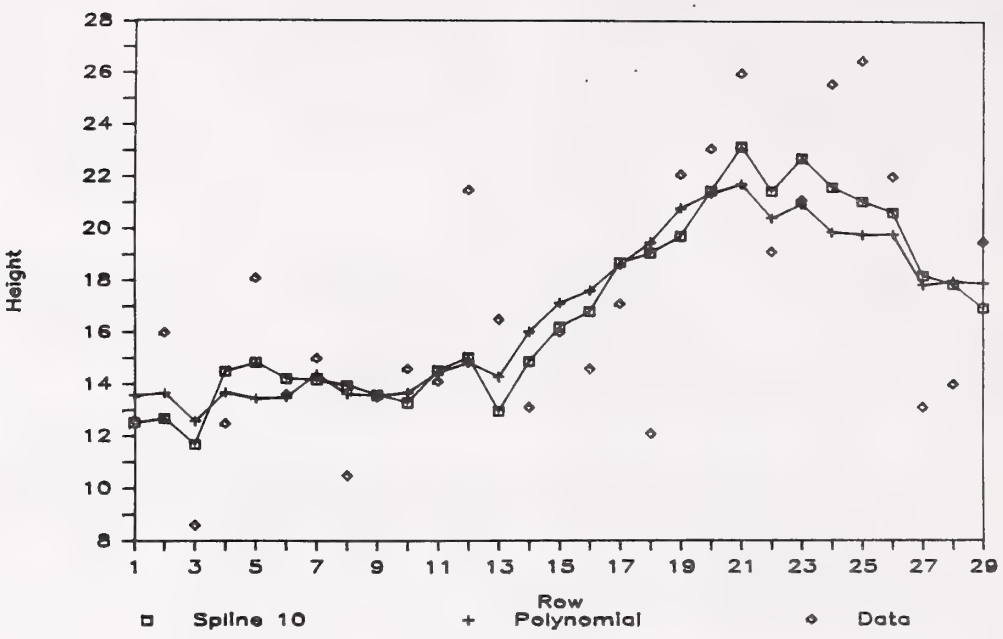


Figure 1. Least-squares spline and polynomial interpolation of environmental trends after one iteration. "10" refers to the number of data per segment.

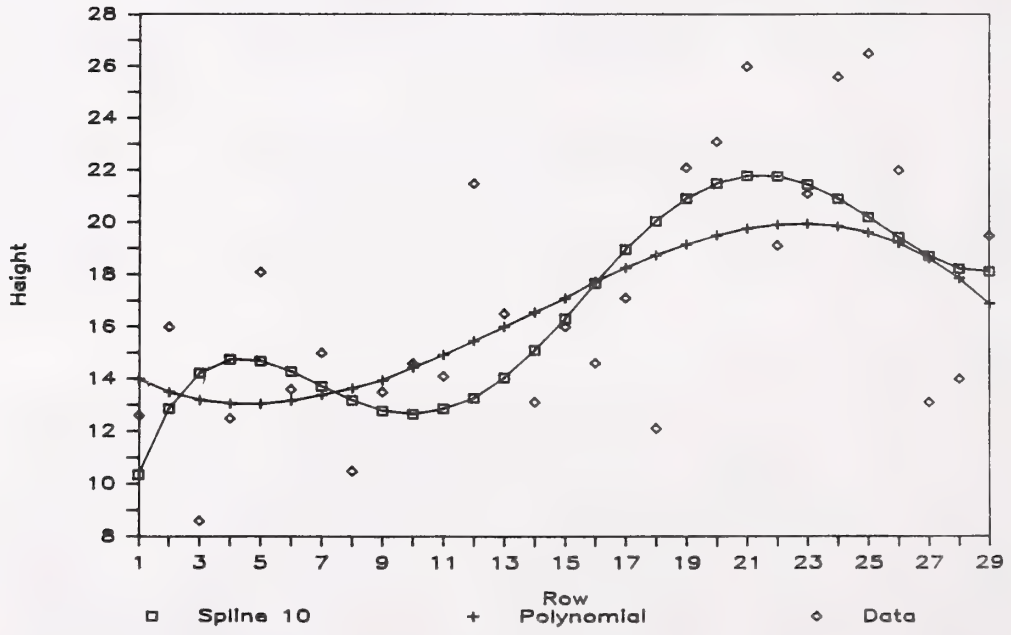


Figure 2. Least-squares spline and polynomial interpolations of environmental trends after ten iterations. "10" refers to the number of data per segment.

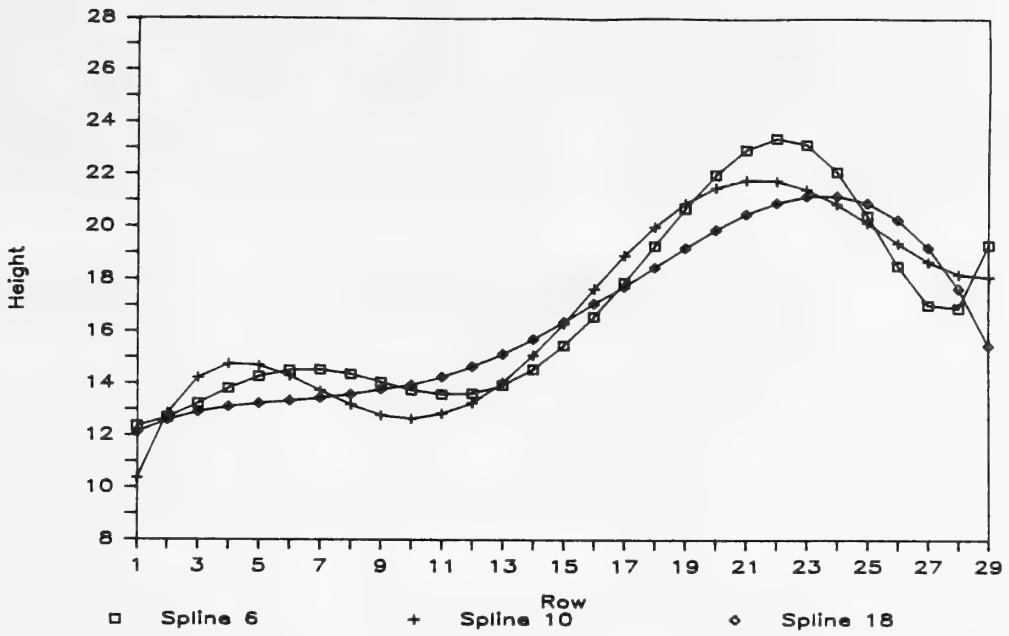


Figure 3. Least-squares spline interpolations of environmental trends after ten iterations using three segment lengths. Numbers refer to the number of plots per segment.

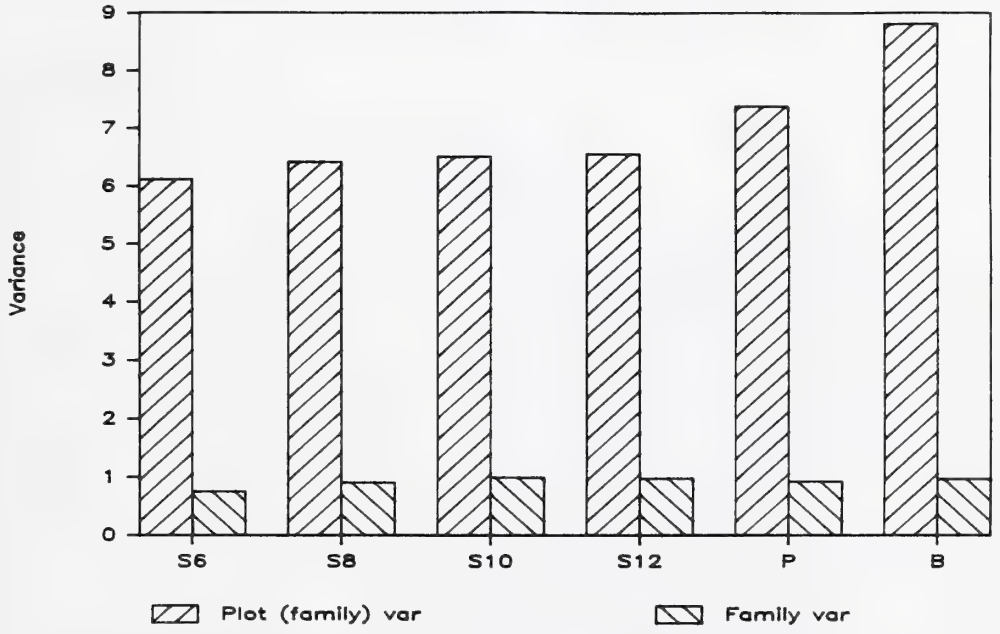


Figure 4. Residual environmental (plot within family) and family variances after environmental gradient effects were removed with splines (S), cubic polynomials (P) and blocking (B). Numbers refer to number of data per segment.

Family variance was also minimized (0.70) with the spline model having the shortest segment length (six plots). As segment length was increased up to ten plots, family variance was also increased. However, further increases had no effect on family variance. With a segment length of ten plots or larger, family variances were approximately the same as with blocking (1.00 with splines vs. 0.98 with blocking). The spline 10 model, therefore, achieved substantial reduction in residual environmental variation without sacrifice of family variation.

The effects on heritabilites are obvious. For both height and diameter, heritabilites were greater when spline models were employed to reduce environmental variation than when blocking was used (Figure 5). For height, the increase was modest: from 0.23 to 0.28. However, for diameter, individual heritability was near zero (0.01) when block effects were eliminated, but was 0.17 when the spline 10 model was used to remove site gradient effects. Furthermore, standard errors of the heritability estimates were somewhat reduced with the spline 10 model (from 0.08 to 0.06 for height, and 0.09 to 0.06 for diameter).

Shortcomings of the blocking technique cannot be attributed to improper placement of the blocks. In the original ANOVA, block effects were highly significant and removed substantial amounts of variation (Table 1). However, becauseenvironmental gradients are not stepwise, family x block interaction will be inflated compared to the true family x environment interaction. The success of the environmental gradient modeling techniques results from their ability to provide closer estimates of the true family x site interation variance (the plot (family) effect in the Table 1 ANOVA).

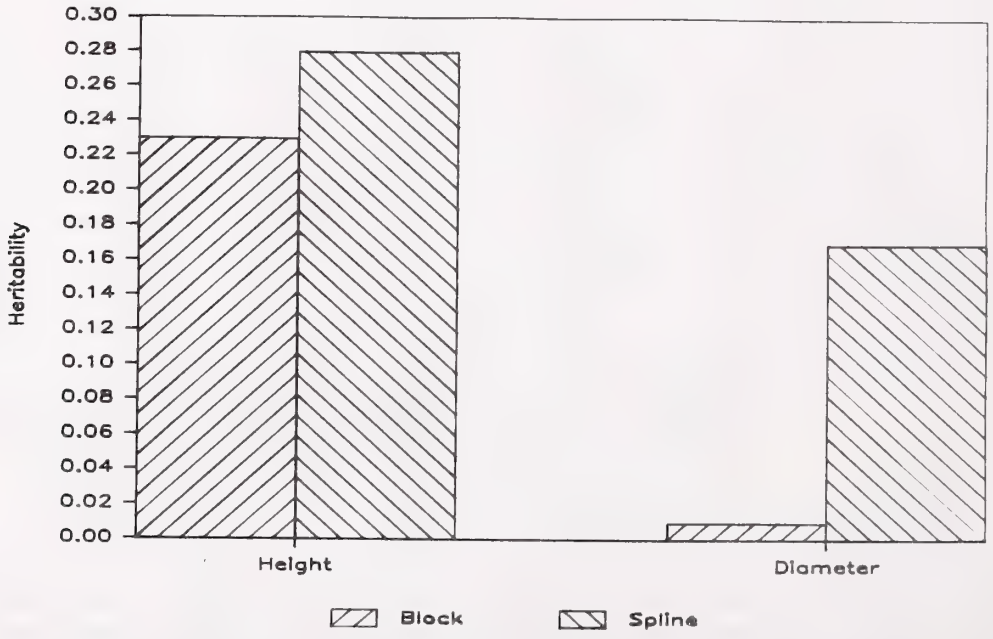


Figure 4. Heritabilities determined after site gradient effects were removed by blocking and by piece-wise least-squares cubic splines with a ten plot segment length.

Table 1. Analysis of variance with blocking and with environmental gradient effects removed by the spline 10 model.

Source	d.f.	Height		Diameter	
		MS	F	MS	F
		with blocking			
Block	3	928.78	138.21**	12.041	74.32**
Family	220	30.70	1.32*	0.573	1.01
Family x block	610	23.25	3.46**	0.565	3.49**
Tree(plot)	790	6.72		0.162	
		with splines			
Family	220	26.71	1.40**	0.525	1.23*
Plot(family)	613	19.11	2.84**	0.426	2.63**
Tree(plot)	790	6.72		0.162	

*Significant at $\alpha = 0.05$.
**Significant at $\alpha = 0.01$.

DISCUSSION

In this paper we have shown one example of how environmental gradient effects may be removed with regression methods (especially least-squares piece-wise splines) better than with blocking. However, the advantages of this approach will vary with site conditions and experimental design. For example, it will be most effective on sites that are environmentally heterogeneous and for traits that are environmentally sensitive. This will almost always be the case in hilly regions and bottomlands. Even in cases where environmental gradients are obvious, blocks are never uniform. Gradients typically occur in more than one dimension, and over distances too small for acceptable blocking.

Once blocking is eliminated from the experimental design, the size of a test need no longer be limited by block size, and the number of allowable genetic entries becomes unlimited. This makes analysis less "messy" and more accurate because test sites may contain the whole complement of families or clones.

Advantages of the spline approach will also be most apparent when single tree plots are employed. Small plots maximize the number of data points from which the model of environmental gradients will be based (only one value per plot is allowable since observations must be genetically random), and should result in the most accurate modeling of these gradients.

Spline models have the advantage of handling border effects well. One or two border rows are probably insufficient to eliminate border effects, but additional rows are too costly. Since least-squares spline models account for border effects, small borders contribute little to environmental variance. However, the trees that comprise border rows must be derived from the same population as the test trees, must be handled in the same manner as the test material and must be measured.

As a final point, the methods describe in this paper are compatible with the statisical and computational equipment presently available. The programs used for this analysis were run in less than two minutes on a personal computer, and the programs are general enough that they can be adapted to most situations with little difficulty.

LITERATURE CITED

de Boor, C. 1978. A practical guide to splines. Springer-Verlag, New York. 392 p.

Namkoong, G. 1979. Introduction to quantitative genetics in forestry USDA, Forest Service Tech. Bull. No. 1588. 342 p.

SAS Institute. 1982. SAS users guide: statistics, 1982 edition. SAS Institute, Inc., Cary, N.C. 584 p.

PROGENY TESTING NITROGEN FIXING TREES PROSOPIS (MESQUITE) AND LEUCAENA FOR BIOMASS PRODUCTION

P. Felker

Abstract. The common mesquite of the American southwest belongs to the nitrogen fixing genus Prosopis which contains 44 species native to North and South America, Africa and the Middle East. In their native habitat the specimens range from 50 cm tall prostrate shrubs to 20 m tall trees with a DBH of 1.4 m. The tree coppices well and the wood has a low volumetric shrinkage (4%) and a specific gravity of 0.7. A Prosopis progeny trial was conducted under heat drought stress in the California Imperial Valley to select material for short rotation energy production. At 2 years of age, fifty five half-sib families ranged from 0.15 to 29 kg dry weight/tree. Clones were made of most productive individual trees. Techniques have since been developed to routinely propagate these clones and to develop efficient cultural practices for their establishment and growth without irrigation. The first clonal plots were harvested in 1986. The standing dry biomass was 3.5 Mg/ha at year 1, 17.6 Mg/ha at year 2 and 39.3 Mg/ha at year 3. Another progeny trial has been completed for the nitrogen fixing tree Leucaena. This trial examined 65 half-sib families of Leucaena leucocephala, the native L. pulverulenta and L. retusa. After 2 seasons growth the biomass of the L.leucocephala and L.pulverulenta families ranged from 650 to 3602 g/tree and 437 to 2505 g/tree respectively. The lone L. retusa accession has a biomass of 290 g/tree. Despite L. retusa's low productivity it has promise since it tolerates -17 C without damage and hybridizes with the more productive species.

Additional keywords: Semi-arid, cold tolerance, coppice, fuelwood.

Nitrogen fixing trees of the genera Prosopis and Leucaena offer considerable promise for semi-arid lands. Generally speaking Leucaena is less well adapted than Prosopis to regions with low rainfall or with freezing weather (Felker et al., 1983; Felker et al 1982). This paper summarizes progeny and clonal

Project Leader, Center for Semi-Arid Forest Resources, Caesar Kleberg Wildlife Research Institute, Texas A&I University, Kingsville, Texas 78363.

biomass production tests conducted in California and Texas for both Leucaena and Prosopis(Glumac et al,1987; Felker et al.,1983; Felker et al., manuscript in preparation).

Leucaena has been primarily viewed as a tropical species with little potential in the United States due to lack of cold tolerance. However Leucaena pulverulenta and Leucaena retusa are native to southern Texas and west Texas respectively and exhibit considerably more cold tolerance than the more widely known Leucaena leucocephala. L. pulverulenta may reach heights over 15 m while the L. retusa seldom reaches heights of 3 m. L. pulverulenta rarely grows farther north than Kingsville Texas (27 30 N) latitude while L. retusa has native populations that exist in far west Texas near Alpine and in southern New Mexico where temperatures reach -18 C. Interspecific hybrids have been made between L. leucocephala and L. pulverulenta and L. retusa. In 1982 a collection of L. pulverulenta half-sib families was made over its naturalized range in south Texas. The productivity of these families were compared to more widely used L. leucocephala species and to a single L. retusa species.

In 1979 a major test of the biomass productivity of Prosopis and other arid adapted genera were examined in a California Imperial Valley field trial (Felker et al.,1983). This site was chosen because of the high daily July maximum temperatures (42 C). This trial compared the biomass productivity of 55 half-sib families that included the genera; Prosopis, Leucaena, Parkinsonia, Cercidium, and Olneya. The dry biomass after 2 years from seeding in the greenhouse ranged from 0.2 kg/tree for Prosopis tamarugo to 29 kg/tree for P. alba (0166). Prosopis alba and P. chilensis from south America were considerably more productive than the California native P. glandulosa var torreyana. The mean dry biomass of 23 California native Prosopis half-sib families ranged from 1.2 to 12.6 kg/tree. Individual Prosopis trees that had a basal diameter greater than 5 cm at the end of the first year were cloned by rooting of cutting techniques.

This paper reports the results of the first field trial using Prosopis clones. This trial had dual objectives of (1) comparing 2 greatly different clones for biomass productivity and (2) determining the absolute productivity for advanced Prosopis selections with good management. One clone B9V18 is a putative P. alba by P. glandulosa var torreyana naturally occurring hybrid and was thorny, and very bushy. Clone B9V18 was selected from the half-sib family P. alba 0166 that had the greatest biomass production of all the families in the California field trial. The female parent of clone B9V18 was thornless and taxonomically was a good specimen of P. alba. The other clone, B2V50 is a Prosopis alba that is nearly thornless. Although clone B2V50 is multistemmed it has a much more erect form than clone B9V18.

METHODS

Sixty-five accessions of L. leucocephala, L. pulverulenta, and L. retusa were planted in March of 1983 on the research plots of Texas A&I University in Kingsville, Texas. A complete description of the origins of these accessions can be found in Glumac et al.,(1987). A randomized complete block design with 4 replicates was used with the 65 accessions. Each replicate consisted of a single row of 5 trees with a 0.5 m in-row and a 1.5 m between row spacing. Thus, 20 trees were evaluated per half-sib family. Each accession was a single tree seed collection (half-sib family) with the exception of the exotics which are mentioned below.

The L. retusa accession came from a tree in Junction, Texas and was provided by D. Ueckert (Texas A&M Research Center, San Angelo, TX). The 38 L. pulverulenta accessions and 5 of the 26 L. leucocephala accessions were collected by Reyes and Felker from trees growing in south Texas during 1982 (Glumac et al 1987). Our accession numbers for named L. leucocephala cultivars are as follows; K4-1086, K8-0147 and 1090 and 1101, K28-0990, K67-0989, K72-1087, K99-1098, K341-1103, Cunningham-1089, and McCarty-1094. The L. leucocephala accessions were obtained from Mark Hutton (CIAT) and sources in Hawaii.

Seeds were hand scarified by nicking, inoculated with Rhizobia (NGR 8) from Nitragin Co., germinated in Ray Leach "supercell" dibble tubes (Canby, Oregon) containing artificial soil mix and grown in a greenhouse for 4 months. The seedlings were handplanted in the field into a raised-bed made with a sub-soiling tree planter. They were planted on a between-row spacing of 1.5 m and an in-row spacing of 0.5 m. Oryzalin (Surflan), a pre-emergent herbicide was incorporated into the soil at the time of planting at a rate of 2.8 kg ha^{-1} and a liquid ammonium phosphate fertilizer (10-34-0) at a rate of 176 kg ha^{-1} was sprayed into the furrow created by the tree planter. The plot was cultivated with a sweep cultivator. Pydrin at a rate of 0.6 kg a.i. ha^{-1} was sprayed on the stems of the trees in October 1984 to control twig girdler (Oncideres pustulata) infestation. A solar powered electric fence was constructed around the plot to prevent browse from wildlife.

Nine months after planting, on December 25 1983, an all time record low temperature of - 12 C occurred. This killed all Leucaena leucocephala and L. pulverulenta accessions to ground level. L. retusa was undamaged by this freeze. Four months after this freeze all of these trees (except L. retusa) were harvested at ground level and weighed. Four entire L. leucocephala and four L. pulverulenta trees were cut into pieces, bagged and dried at 50°C for moisture content determinations. This biomass is listed in Table 1 as biomass

after 9 months. Many of the L. leucocephala were over 3 m tall at the time of this freeze.

Nearly all of these trees resprouted from the stump and were 3 to 4 m tall by the next winter. During the winter of 1984/1985 temperatures of - 9 C occurred which again killed all of the L. leucocephala to ground level and which caused varying degrees of stem kill to the L. pulverulenta. An evaluation of the extent of the freeze damage was made in May 1985 after new growth occurred and the position of stem kill was firmly established. The greatest stem dieback (distance from tip to regrowth) on each tree and height of the stem was measured. The mean ratio of stem dieback to height was calculated for each accession (1 = 100 % dieback). Also in May 1985 the number of surviving rootstocks and the biomass of the resprouts was measured with regressions as previously described(Glumac et al.1987).

A means separation of the biomass measurements conducted with Tukey's HSD test indicated the 65 accessions could be grouped into about 8 significant groups. As the reporting of this grouping was cumbersome and not very useful in practical terms, only the standard errors of the mean biomass and freeze dieback ratios are reported.

Prosopis clonal biomass production trial

A completely randomized design was used in which 3 plots of P. alba clone B2V50 were harvested at the end of 1, 2, and 3 years growth. At the end of 3 year's growth, 3 plots of the hybrid clone P. alba X P. glandulosa var torreyana were also harvested. Each sample plot consisted of 25 trees on a 3m x 3m spacing for a plot size of 225 m^2. The sample plots for biomass estimation were surrounded by 2 border rows. Due to the difficulty in making clones, the most productive Prosopis from seed i.e. P. alba 0166 was used for the border rows. In the second and third growing season, the biomass of the P. alba 0166 border row closest to the measurement plots was also determined.

Site preparation for this trial began in the fall of 1983. After small trees were uprooted, the site was mowed, sprayed with glyphosate to kill perennial grasses, mouldboard plowed and disked several times. Rooted cuttings were made as previously described (Klass et al., 1985) and transplanted into 38 cm long plant bands. The rooted cuttings were transplanted into the field on March 16, 1984 using a previously described mechanical tree planter (Felker et al, 1984) modified from a subsoiler. The trees were planted in a rectangular grid pattern to allow cultivation perpendicular to the direction of planting. Two days prior to planting, oryzalin was applied at a rate of 2.4 kg a.i./ha. No irrigation was used at any phase of the planting. After planting, a 50 cm tall chickenwire fence was placed around the planting to reduce rabbit browse.

The plots received no rain either 6 weeks prior to planting or 8 weeks after planting. Additionally temperatures of 38.8 C (102 F) and 40.5 C (105F) occurred after the planting but before the rains. In spite of the adverse conditions only 3 trees out of 225 had to be replaced (98.7% survival). No leaf loss or other sign of stress appeared on the other trees. The high survival is attributable to deep plowing during the previous rainy season and the long cardboard plant bands.

An intensive weed control program was used. These plots were mechanically cultivated using disk harrows and sweep cultivators several times a year for each of the 3 years. Cultivation was carried in the direction of the rows and across the rows. At the end of the first and second growing season the herbicide solicam was applied at 5 kg/ha and effectively controlled nutsedge, johnsongrass and bermuda grass. Three plots of 25 trees/plot were harvested December 6-7, 1984, November 26 and 27 1985, and in December 1986. For dry matter determinations, entire trees were reduced in size and dried at 70 C until equilibrium was reached.

RESULTS

Leucaena trial

The mean dry biomass per tree for 9 months growth after planting (April 1984 harvest of freeze killed trees) and for the second year's regrowth after the freeze (April 1985 regression estimation) is shown in Table 1. The accessions are ranked according to the highest mean for the 2nd year estimates.

The biomass of the L. leucocephala half-sib families ranged from 3602 g/tree for accession 1090 to 654 g/tree for accession 1099. The biomass of the L pulverulenta half-sibs ranged from 2505 g/tree for accession 0999 to 437 g/tree for accession 1041. The lone L. retusa had the lowest biomass productivity of 290 g/tree. Thus there was considerable overlap in productivity between the species L. pulverulenta and L. leucocephala depending upon which family was examined.

An opposite trend was observed in freezing tolerance with all of the L. leucocephala having a freeze dieback ratio of 1.00 (100 % dieback), with L. pulverulenta ranging from 0.07 to 0.37 and with the L. retusa having a freeze dieback ratio of 0.00.

The five most productive accessions were L. leucocephala K8(2 sources), K72, K67 and a local strain from Zapata TX. The 3 most productive L. pulverulenta accessions were from Kingsville (0999) and Harlingen (1046 & 1047). Another productive accession was 1094 from a tree growing at the University of Florida. The standard errors for biomass productivity of the L. pulverulenta are generally greater than the L. leucocephala. This is presumably due to the fact that L. leucocephala is

highly self fertile and inbred while the other specie's has a greater tendency to outcross.

Table 1. A comparison of biomass production, cold tolerance and survival of Leucaena leucocephala, Leucaena pulverulenta and Leucaena retusa.

Accession	9 month growth after planting mean ± SE** (g)		2nd year regrowth after freeze mean ± SE** (g)		Freeze dieback ratio mean ± SE**		% survival 1983-85 (n=20)
1090 L	1998	149	3602	280	1.00	0.00	95
1071 L	2009	326	3169	474	1.00	0.00	95
1087 L	1585	185	2866	910	1.00	0.00	65*
0147 L	1928	229	2784	152	1.00	0.00	75*
0989 L	1527	282	2780	396	1.00	0.00	90
1094 L	1657	130	2645	318	1.00	0.00	100
1086 L	1494	150	2645	223	1.00	0.00	75
0999 P	1051	383	2505	1041	0.19	0.07	75
1046 P	727	338	2381	763	0.08	0.01	65*
1047 P	635	140	2219	758	0.20	0.06	80*
1044 P	1090	564	2164	766	0.18	0.03	65*
1092 L	1207	166	2119	488	1.00	0.00	100
1096 L	718	362	2117	434	1.00	0.00	75
1002 P	879	190	2020	731	0.19	0.05	75
1005 P	653	270	2020	622	0.14	0.08	50*
1073 P	1057	230	2017	236	0.09	0.02	95
1091 L	910	315	1953	649	1.00	0.00	60*
1056 P	732	321	1878	540	0.08	0.01	70
0993 L	1113	233	1868	471	1.00	0.00	60*
1060 L	1384	93	1806	298	1.00	0.00	100
1103 L	1340	211	1793	466	1.00	0.00	90
1036 P	703	259	1780	548	0.15	0.04	90
1001 P	865	84	1751	225	0.11	0.02	95
1050 P	733	382	1732	975	0.19	0.05	65
1070 L	1007	266	1728	412	1.00	0.00	75*
1067 P	895	197	1710	606	0.49	0.12	75
1088 L	1390	249	1710	307	1.00	0.00	75
1101 L	818	288	1614	330	1.00	0.00	65*
1089 L	905	185	1610	523	1.00	0.00	85
1052 L	815	278	1602	73	1.00	0.00	75*
1097 L	1009	260	1590	285	1.00	0.00	80
1069 P	875	54	1566	375	0.07	0.01	70
1078 P	715	79	1563	411	0.09	0.02	70*
1085 L	916	137	1506	217	1.00	0.00	90
1059 P	674	250	1444	556	0.25	0.07	70
1045 P	616	163	1404	318	0.18	0.03	90
1043 P	553	111	1358	303	0.22	0.04	90
1003 P	512	76	1330	201	0.22	0.04	60
1098 L	774	177	1292	374	1.00	0.00	100

Table 1. Continued

Accession	9 month growth after planting mean ± SE** (g)		2nd year regrowth after freeze mean ± SE** (g)		Freeze dieback ratio mean ± SE**		% survival 1983-85 (n=20)
1035 P	851	149	1281	166	0.29	0.07	95
1074 P	684	96	1269	376	0.14	0.09	80
1065 P	540	107	1267	56	0.14	0.06	75
1100 L	493	102	1203	622	1.00	0.00	85
1081 P	556	166	1189	310	0.17	0.06	80
1004 P	529	166	1180	250	0.18	0.03	90
1038 P	704	171	1173	359	0.16	0.05	90
1079 P	541	228	1138	379	0.26	0.08	65*
0990 L	616	127	1137	377	1.00	0.00	75
1064 P	548	201	1078	239	0.27	0.00	85
1000 L	848	129	1060	337	0.18	0.02	90
1055 P	470	164	943	309	0.23	0.07	70
1080 P	376	141	920	373	0.21	0.07	80
1076 P	265	129	870	274	0.15	0.02	45*
1077 P	364	51	816	296	0.19	0.08	75
1075 P	560	254	815	277	1.00	0.00	60
1095 L	676	111	785	126	1.00	0.00	90
1066 P	608	253	707	131	0.08	0.02	80
1061 P	432	185	691	249	0.13	0.05	85
1099 L	645	246	654	333	1.00	0.00	80
1051 P	358	55	577	70	0.32	0.08	80
1037 P	359	104	569	106	0.21	0.05	65
1042 P	181	36	537	52	0.37	0.07	60
1040 P	181	47	450	97	0.24	0.07	70
1041 P	134	16	437	136	0.34	0.06	60
1084 R	***	***	290	***	0.00	0.00	5

L=*L. leucocephala* P=*L. pulverulenta* R=*L. retusa*. Freeze dieback ratio is stem dieback/height of stem. Survival based on number of trees still alive out of 20 on May 8, 1985.

* Accession was located in area of block 4 where damage was incurred from the herbicide bromacil

** Mean and standard errors are based on a maximum of 20 trees. The sample size for each accession can be derived by dividing the corresponding percent survival (1983-85) by 5.

*** *L. retusa* was not harvested and was represented by 1 tree.

RESULTS

The biomass productivity of clone B2V50, B9V18 and associated seed propagated border rows is presented in Table 2. *Prosopis alba* clone B2V50 had a standing dry weight of 3.5 Mg/ha, 17.6 Mg/ha, and 39.3 Mg/ha at the end of the first, second and third year's growth respectively. The high productivity of 21.7 Mg/ha for the third growing season is especially promising. *Prosopis*

alba by P. glandulosa var. torreyana clone B9V18 had a mean standing dry biomass 16.6 ± 4.2 Mg/ha.

It is interesting to compare the productivity of clones B2V50 and B9V18 to the border row of P. alba 0166. As an average, clone B2V50 had 74 % greater biomass than the adjacent border row of seed propagated P. alba 0166 trees. In contrast clone B9V18 had 35 % less fresh weight than the border rows. Thus in this case a decrease in productivity was observed by cloning an individual within a family. In contrast, P. alba clone B2V50 yielded 74 % greater biomass than the best seed propagated material available to date.

Table 2 Biomass production of clone B2V50 at 1, 2 and 3 seasons growth.

		Mean Tree fresh wt. (kg)	Standing dry weight (Mg/ha)	Annual growth (Mg/ha)
		CLONE B2V50		
Year 1	inner B2V50	7.3 ± 0.7	3.5 ± 0.3	3.5
Year 2	inner B2V50	31.4 ± 2.2	17.6 ± 1.2	14.1
	outer 0166	25.0 ± 4.7		
Year 3	inner B2V50	65.4 ± 12.6	39.3 ± 7.6	21.7
	outer 0166	37.4 ± 12.8		
		CLONE B9V18		
Year 3				
	inner B9V18	25.1 ± 6.5	16.6 ± 4.2	
	outer 0166	38.7 ± 14.2		

DISCUSSION

Leucaena leucocephala has grown 3 m in height from seedlings the first year and one year coppice regrowth has been 5 m tall with a biomass production of 14 dry Mg/ha (Glumac et al, 1987). A 6 fold variation in biomass production was observed for L. leucocephala. Natural populations of Leucaena pulverulenta had 5 fold variation in growth rate and a 4 fold variation in the percent freeze dieback. Nevertheless Leucaena pulverulenta is not sufficiently cold tolerant to be adaptable to major regions of southeastern United States. However L. retusa has survived -18 C (0 F) without damage and has been reported to form interspecific hybrids with the other species in this study (Brewbaker pers. comm.) The possibility of developing a cold tolerant Leucaena species to the low fertility conifer sites of southeastern United States is truly exciting.

Major strides have been made in the last 10 years in identifying and clonally propagating superior Prosopis phenotypes for biofuel production (Felker et al 1983), salt tolerance (Rhodes and Felker 1987, and pod production (Oduol et al, 1986). However the selection process used less than 10 non-related half-sib families of Prosopis alba and only 1 half-sib family of Prosopis chilensis. Funding sources need to be identified to permit a broader evaluation of this genus to reduce inbreeding problems, to reduce the genetic vulnerability of the clones, and to identify more productive genotypes with greater cold hardiness.

LITERATURE CITED

Felker,P., P. R. Clark, P. N., J.F. Osborn, and G. H. Cannell. 1981. Screening Prosopis (mesquite) for cold tolerance. Forest Science. 28: 556-562.

Felker,P., G.H. Cannell, P.R. Clark, J.F. Osborn, and P. Nash. 1982. Biomass production of Prosopis species(mesquite), Leucaena, and other leguminous trees grown under heat/drought stress. Forest Science. 29:592-606.

Felker, P., D. Smith, M. Smith, R.L. Bingham and I. Reyes. 1984 Evaluation of herbicides for use in transplanting Leucaena leucocephala and Prosopis alba on semi-arid lands without irrigation. Forest Science. 30:747-755.

Klass, S.,; R.L. Bingham, L. Finkner-Templeman and P. Felker. 1984. Optimizing the environment for rooting cuttings of highly productive clones of Prosopis alba (mesquite/algarrobo). Journal of Horticultural Science 60:275-284.

Oduol, P. A., P. Felker, C. R. McKinley and C.E. Meier. 1986. Variation among selected Prosopis families for pod sugar and pod protein contents. Forest Ecology and Management 16:423-431.

Glumac,E.L., P. Felker and I. Reyes. 1987. A comparison of cold tolerance and biomass production in Leucaena leucocephala, Leucaena pulverulenta, and Leucaena retusa. Forest Ecology and Management in press

Rhodes, D. and P. Felker 1987. Mass screening Prosopis (mesquite) seedlings for growth at seawater salinity. Forest Ecology and Management.in press

A SEED SOURCE AND PROGENY TEST OF SELECT EAST COAST LOBLOLLY PINES IN ARKANSAS AND MISSISSIPPI

R. C. Schmidtling[1/]

Abstract.--This test contrasts a collection of half-sib loblolly families from orchards in the coastal plain of the Carolinas with woods-run seed from the Carolinas and Arkansas and orchard seed from Arkansas.

The three plantings are located in southern Arkansas and northern Mississippi and consist of up to 18 North Carolina families, 21 South Carolina families, a bulk collection from a southeast Arkansas orchard, and woods run checks from North Carolina, South Carolina, and Arkansas. They are planted in 64-tree plots.

After 13 years, although there was a strong location x family interaction, the Atlantic coast selections were outgrowing woods-run trees of Carolina or Arkansas origin and also were outgrowing the Arkansas orchard trees in the Arkansas planting. The advantage of the coastal sources was not as clear at the Mississippi planting after 11 years.

These results so far confirm the value of Carolina Coastal Plain loblolly for use in Arkansas.

Conclusions from the Southwide Pine Seed Source Study (SPSSS) and other provenance tests (Kraus et al. 1984, Switzer and Wells 1964, Wells and Wakely 1966, Wells and Lambeth 1983) have resulted in large-scale movement of loblolly pine seed in two opposite directions.

The first of these is the eastward movement of Livingston Parish, Louisiana, sources (Wells 1985) and more recently, Texas sources to high fusiform rust hazard sites in Florida, Georgia, Alabama and South Carolina. This has been effective in reducing rust infection in these areas.

The other large-scale movement of a provenance, which is more pertinent to the present paper, involves the westward movement of loblolly sources from the coastal plain of North and South Carolina to Arkansas and Oklahoma. In Southern Arkansas, the local seed source was the poorest growing provenance in the loblolly SPSSS and the Onslow, North Carolina, source was the best growing source (Wells 1983). A more specialized provenance test in south Arkansas

---

1/ Principal Plant Geneticist, USDA-Forest Service, Southern Forest Experiment Station, P. O. Box 2008, GMF, Gulfport, Mississippi 39505. The author is grateful for help received from many cooperators. The following members of the North Carolina State University Cooperative Tree Improvement Program, under the direction of Dr. Bruce Zobel, provided seed for the study: International Paper Company, Weyerhaeuser Company, Riegel Paper Corporation, (now Federal Paper Board Company) Westvaco Corporation, and the South Carolina Commission of Forestry. Additional seed was contributed by the Francis Marion National Forest. Planting sites, installation, maintenance, and measurement assistance were provided by the Crossett Division of Georgia-Pacific Corporation (now Rex Timber, Inc.) and the Dierks Division of Weyerhaeuser Company. The study was initiated by Hoy Grigsby; planting and early data collection supervised by O. O. Wells (both USDA-Forest Service, Retired).

confirmed the superiority of the coastal North Carolina sources over the local south Arkansas source (Wells and Lambeth 1983). As a result, large quantities of North Carolina loblolly have been planted in Arkansas and Oklahoma (Lambeth et al. 1984).

The present study seeks to extend the previous results by determining the growth of progeny from selected North and South Carolina coastal plain parent trees relative to woods-run Carolina coastal plain and select and woods-run Arkansas trees when planted in south Arkansas and north Mississippi.

MATERIALS AND METHODS

Orchard-pollinated seed was collected from plus-tree selections from the North Carolina State University Tree Improvement Cooperative and the Southern Region of the U. S. Forest Service (figure 1). Several check lots were also included: North Carolina coastal woods-run, South Carolina coastal woods-run, Southeast Arkansas woods-run, and South Arkansas plus-tree mix. Another check lot, Mississippi-Alabama woods-run was used in the Mississippi planting in place of the South Carolina woods-run.

The seed were stratified and sown in a nursery near Crossett, Arkansas. One-year-old seedlings were bar-planted at two locations in Arkansas in 1974, and in one location in Mississippi in 1976 (figure 1). The plots were 64 trees square with border rows around the outside perimeter only. Spacing was 8-by 8-feet.

Eighteen North Carolina families and 20 South Carolina families plus checks were planted at the Crossett location. Only 15 of the same 18 North Carolina selections and 17 of the 20 South Carolinas families were planted at the Horatio location.

The Stewart location was planted 2 years later and consisted of 14 North Carolina selections and 13 South Carolina selections plus checks. Nine of the 14 North Carolina selections and 12 of the 13 South Carolina selections had been included in the other two plantings.

All three plantings were on flat coastal plain sites. The Mississippi planting was on a well-drained soil, the Arkansas plantings were on poorly to somewhat poorly drained soils (table 1). Five replications were planted at each location.

Survival was tallied at age 1 for the Arkansas plantings and age 3 for the Mississippi planting. Heights were measured and fusiform rust infection was tallied at all three plantings at age 5.

In late summer of 1986, a southern pine beetle infestation was found in the Crossett planting. In fall of 1986, all three plantings were measured prior to the salvage operation at Crossett. Remeasurements of DBH were made on all trees, and height was measured on a 20 percent random sample from each plot. Evidence of beetle infestation was also tallied at the Crossett location.

A regression formula was constructed for each planting to estimate heights of the non-sample trees from their DBH measurements. Plot volumes were estimated using a volume formula for young plantation loblolly (Schmitt and Bower 1970). Analysis of variance (SAS GLM procedure) was used to test differences in means.

Survival, height, basal area, volume, and rust infection were analyzed separately for each planting. An analysis was also done on the combined data for Horatio and Crossett, after deleting the six families from the Crossett data set which were not included in the Horatio planting.

RESULTS AND DISCUSSION

Survival

Most of the mortality in all three plantings occurred shortly after planting. Survival at 3 years averaged 59 percent at Horatio and 75 percent at Crossett. Survival after 1 year was 89 percent at Stewart. In fall of 1986, survival averaged 57 percent at Horatio, 69 percent at Crossett and 85 percent at Stewart.

The variation in survival among plantings generally follows what would be expected because of the increasing levels of moisture stress from the eastern to the western planting (figure 1). At the Horatio planting nearly 20 percent of the years would be expected to have less precipitation than evaporation according to Visher's (1954) atlas. The Mississippi planting is in a zone where yearly evaporation would never be expected to exceed rainfall. In addition, the Mississippi planting is on a well drained site, where trees should be under less stress because of better root developement than at the poorly-drained Arkansas plantings.

Survival of the local sources was no better, and sometimes worse, than the Carolina coastal plain sources. Survival of the Arkansas woods-run trees averaged only 32 percent at the Horatio site compared to 78 percent for North Carolina woods-run trees. The Arkansas sources survived better at the Crossett site (53%), but did not survive as well as the other sources (75%). The Arkansas woods-run sources survived the best at the Stewart site, although there was not much difference amoung sources.

Family differences in survival were significant in all plantings. In the combined Horatio-Crossett data, location x family interaction was significant and large. Survival of families and sources was not consistent across the three plantings.

Height growth

Results at the Horatio planting approximate those which would have been anticipated, based on the SPSSS and other provenance and progeny tests. The Arkansas woods-run source was last, both in height growth at 33.9 feet and volume (table 1). The SC and NC woods-run sources averaged 3 to 5 feet taller than the Arkansas woods run. The Arkansas plus-tree source averaged nearly 4 feet taller than the Arkansas woods-run, and was taller than the SC woods run trees. Most of the NC and SC plus tree families performed better than the NC check; all but one performed better than the SC check.

In the combined Horatio-Crossett analysis, the location x source interaction was significant for height growth. This is readily apparent in comparing the relative rankings in the two plantings in table 1. The ranking of the Arkansas woods-run at the Crossett location was similar to it's ranking at Horatio: near the bottom, at 37.9 feet in height. The Arkansas plus-tree, selections, however, were not any better than the checks at the Crossett location and actually averaged slightly shorter. At the Horatio location, the NC and SC woods-run sources exchanged places in rankings relative to the Crossett location. The NC woods-run trees are second from the bottom at Horatio, averaging 0.3 feet shorter than the Arkansas woods run.

The SC and NC select trees did perform well relative to all the checks at the Crossett location as well as at the Horatio location, but there are many examples of changes in rank when comparing Crossett with Horatio. There are also some examples of genetic stability. For instance, SC 11-10 and SC 11-25 rank second and third in height at both locations.

At the Stewart location, the relative performance of the Arkansas sources was better than in the two Arkansas plantings (table 2). The Arkansas plus-trees were above average in height, the Arkansas woods-run were just below average, along with the MS-AL local woods-run sources. The NC woods-run source was shorter by 0.3 feet than the Arkansas woods-run, but this difference is small compared to the standard error.

The relatively good performance of the non-local Arkansas sources in Mississippi is surprising, considering how poorly they did as a local source in Arkansas. These results perhaps could have been expected, however, considering the results of the SPSSS at the two locations (Wells and Wakeley 1966). In the Clark County, Arkansas SPSSS planting the local Arkansas source was fourteenth out of 15 sources in height growth; the coastal North Carolina source was first. In the Winston County, Mississippi, planting of the SPSSS, however, the Arkansas source was not significantly shorter than the North Carolina source.

The variation in soils among the three locations may also be a factor in the difference in relative performance among locations. The Arkansas plantings are on poorly drained, or somewhat poorly drained, soils which is more similar to the soils on the Carolina coastal plain than the well-drained soils of the Mississippi planting.

Volume

Plot volume was not closely related to height growth, since at this age volume is heavily dependent on initial survival. The poor survival of the Arkansas sources in Arkansas is reflected by their low plot volumes, however. At Horatio, the Arkansas woods-run ranks last in volume as well as height (table 1). The Arkansas plus-tree source is slightly below average in volume as well as height. At the Crossett location the Arkansas woods-run and plus-tree selections rank fortieth and forty-second, respectively, out of 42 sources in volume.

At the Stewart location, the Arkansas plus-tree and woods-run sources are at or slightly above average in volume, in line with their height growth ranking.

Volume measurements will be more definitive in a few years, when the plots reach culmination. There is already evidence for some compensation in diameter growth for low stocking levels, as average diameter and survival are negatively correlated on a plot mean basis ($r = -0.59^{**}$, Crossett planting). There is also some evidence for self thinning in the plots with the best survival. Losses subsequent to the 3 year measurements have been negligible on plots with poor initial survival, but amount to up to 15 percent on plots with good survival.

Southern Pine Beetle

The measurements of these plantings were scheduled in fall of 1986 mainly because of a southern pine beetle infestation in the Crossett planting. The outbreak was primarily in a 1-acre area at the head of a draw, and appeared to have originated outside the planting. The plot with the most damage was a North Carolina Selection, 8-131, where most of the trees were dead. The infestation was related to location rather than genetics, however. All heavily infested plots were adjacent to each other, and out of eight plots which had beetle-killed trees, two were local sources: an Arkansas woods-run

plot and an Arkansas plus-tree plot. This would seem to be evidence that the Carolina sources are not any more susceptible (at age 13) than local sources, since only 2 out of 42 sources are local.

Rust Infection

Fusiform rust infection at the two Arkansas plantings was negligible, and could not be analyzed. Rust infection at the Stewart location averaged about 13 percent, and there were significant differences amoung sources (table 2). As expected, the Arkansas sources were only lightly infected, 3.2 percent for the Arkansas checks and 6.6 percent for the Arkansas plus-trees. There was wide variation in rust infection among the coastal plain families, ranging from 2 percent for family SC 11-16 to 37 percent for family SC 7-58.

CONCLUSIONS

The poor growth rate of the Arkansas sources in Arkansas is difficult to explain. In the loblolly SPSSS, the western sources survived better (Wells and Wakeley 1966) and it was concluded that the western sources, though slower growing, had a survival advantage under the stressful conditions in the western part of the loblolly range. Lambeth et al. (1984), did not find that the Arkansas sources were clearly superior in early survival to coastal Carolina sources in Arkansas and Oklahoma. This was also not true in this test in Arkansas. In both Arkansas plantings survival of the Arkansas woods-run and the Arkansas plus-tree sources was below average. In the Horatio planting, the Arkansas woods-run ranked last in survival, as well as height and volume. The natural regeneration situation is quite different from that of bar-planting 1-0 stock on intensively site prepared land however, and under natural conditions the Arkansas sources may have a clear survival advantage.

There is some evidence for mortality in coastal sources planted in Arkansas due to moisture stress late in the rotation (Lambeth et al. 1984). This has not been the case in the Loblolly SPSSS planting in south Arkansas after 30 years.[1/]

There is undoubtedly some risk associated with planting coastal Carolina sources in Arkansas, but the record drought of summer 1980 and the record cold of December 1984 did not appear to affect these plantings adversely. Planting improved Carolina Coastal Plain loblolly in southern Arkansas seems to be justified based on these results. Planting these same sources in North Mississippi seems to be less advantageous.

LITERATURE CITED

Kraus, John F., O. O. Wells and E. R. Sluder. 1985. Review of provenance variation in loblolly pine (Pinus taeda L.) in the southern United States. In: Barnes, R. C., and G. L. Gibson, eds. Proceedings of a Joint Work Conference on Provenance and Genetic Improvement Strategies in Tropical Forest Trees; 1984 April 9-14; Mutare, Zimbabwe: Oxford England, Commonwealth Forestry Institute; 281-317.

1/ Schmidtling, R. C. unpublished 30 year data from the loblolly SPSSS.

Lambeth, C. C., P. M. Dougherty, W. T. Gladstone, R. B. McCullough and O. O. Wells. 1984. Large-scale planting of North Carolina loblolly pine in Arkansas and Oklahoma: A case of gain versus risk. J. For. 82:736-741.

Schmitt, Dan and Dave Bower. 1970. Volume tables for young loblolly, slash, and longleaf pines in plantations in south Mississippi. Res. Note SO-102. New Orleans, LA: U.S. Department of Agriculture, Forest Service. 6 p.

G. L. Switzer and O. O. Wells. 1964. Geographic seed origin and early performance of loblolly pine on the Northeast Mississippi Experimental Forest. Mississippi State University Information Sheet 850. Mississippi State University Agricultural Experiment Station, State College, MS. 2 p.

Visher, S. S. 1954. Climatic Atlas of the United States. Harvard University Press, Cambridge, Mass. 403 p.

Wells, O. O. 1983. Southwide pine seed source study--loblolly pine at 25 years. South. Jour. of Appl. For. 7(2):63-70.

Wells, O. O. 1985. Use of Livingston Parish, Louisiana, loblolly pine by forest products industries in the Southeast. South. Jour. of Appl. For. 9(3):180-185.

Wells, O. O. and C. C. Lambeth. 1983. Loblolly pine provenance test in southern Arkansas 25th year results. South. Jour. Appl. For. 7(2):71-75.

Wells, O. O. and P. C. Wakeley. 1966. Geographic variation in survival, growth, and fusiform-rust infection of planted loblolly pine. For. Sci. Mono. 11. 40 p.

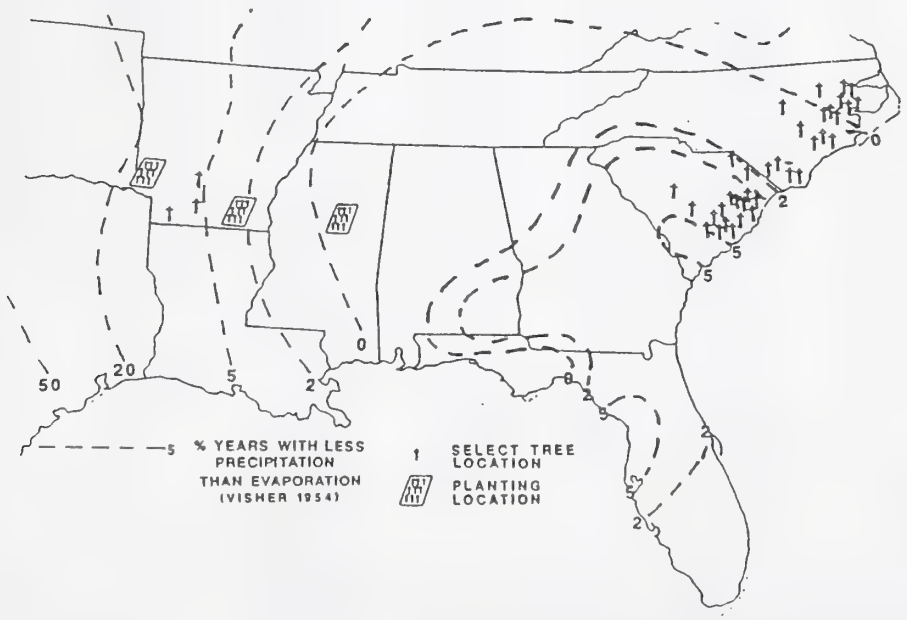


Figure 1. Map of Southeastern U. S. showing location of plantings and seed sources. Dashed lines are adapted from Visher 1954 and show the percentage of years in which precipitation is less than evaporation; used here as an indication of droughtiness.

Table 1.--Family means for Plot volume and height growth of select Carolina coastal families and checks planted in south Arkansas. They are ranked by average height.

Horatio AR Planting						Crossett AR Planting			
Family	Ht ft	SE [1]	Plot Vol Cu Ft	Rank	SE [1]	Family	Ht ft	Plot Vol Cu Ft	Rank
NC 6-26	41.0	1.2	230	(2)	18	SC 7-34	42.5	194	(9)
SC 11-10	40.7	1.2	210	(5)	18	SC 11-10	42.4	164	(28)
SC 11-25	40.9	1.2	195	(9)	18	SC 11-25	42.4	185	(13)
NC 7-29	40.4	1.2	185	(13)	18	SC 11-9	42.1	181	(17)
SC 18-117	40.0	1.0	239	(1)	16	SC 7-56	41.6	170	(27)
NC 9-6	40.0	1.2	147	(23)	18	SC F-67	41.4	228	(1)
NC 8-74	40.0	1.0	168	(18)	16	SC F-39	41.2	198	(6)
SC 7-4	39.9	1.2	194	(10)	18	SC 7-2	41.1	185	(15)
SC 11-36	39.8	1.2	224	(3)	18	NC 7-29	41.1	173	(26)
NC 7-88	39.8	1.2	150	(22)	18	SC 11-22	41.1	187	(12)
SC 11-21	39.7	1.2	213	(4)	18	NC 8-103	41.1	161	(29)
NC 7-60	39.7	1.2	177	(15)	18	SC 11-16	41.0	152	(32)
SC 7-2	39.7	1.0	142	(27)	16	NC 7-60	40.9	193	(10)
NC 8-131	39.6	1.2	201	(6)	18	SC F-209	40.9	199	(5)
SC 7-34	39.6	1.0	168	(16)	16	NC 7-62	40.9	177	(23)
SC F-209	39.2	1.0	168	(17)	16	NC 8-74	40.8	204	(3)
NC 8-103	39.1	1.0	134	(30)	16	SC 11-21	40.7	203	(4)
SC F-39	39.1	1.2	192	(12)	18	NC 9-3	40.7	195	(8)
SC 11-26	39.0	1.0	156	(21)	16	NC 9-6	40.7	158	(30)
NC Check	38.9	1.2	199	(8)	18	NC 8-131	40.5	178	(22)
NC 7-59	38.8	1.2	144	(24)	18	SC 7-4	40.5	185	(14)
SC 7-56	38.5	1.2	116	(33)	18	NC 8-136	40.4	137	(38)
SC 7-58	38.4	1.2	167	(19)	18	NC 8-30	40.2	178	(21)
SC 11-9	38.2	1.0	162	(20)	16	SC 18-34	40.1	179	(20)
SC 18-102	38.2	1.2	200	(7)	18	NC 7-88	39.7	149	(35)
NC 7-90	38.0	1.5	137	(28)	22	NC 7-59	39.5	151	(33)
AR + Tree	37.8	1.2	137	(29)	18	SC 18-117	39.5	219	(2)
SC 18-34	37.8	1.0	194	(11)	16	SC 11-41	39.4	192	(11)
NC 8-30	37.6	1.5	180	(14)	22	SC 7-58	39.4	174	(25)
NC 8-35	37.6	1.0	144	(26)	16	NC 6-26	39.2	179	(18)
NC 7-62	37.6	1.0	106	(34)	16	SC Check	39.1	156	(31)
NC 8-136	37.5	1.2	132	(31)	18	SC 18-102	39.0	182	(16)
SC 11-16	37.4	1.0	102	(35)	16	NC 7-90	38.9	147	(36)
SC Check	37.2	1.0	144	(25)	16	NC 8-35	38.8	151	(34)
NC 8-27	37.2	1.2	126	(32)	18	SC 11-26	38.6	179	(19)
AR Check	33.9	1.2	88	(36)	18	SC 11-36	38.2	195	(7)
						NC 8-27	38.1	123	(41)
						AR Check	37.9	130	(40)
						AR + Tree	37.7	112	(42)
						NC 9-4	37.7	136	(39)
						NC Check	37.6	177	(24)
						NC 8-46	36.8	138	(37)
Mean	38.9		166			Mean	40.0	173	
						St. error	0.8	10	

[1]SE=Standard errors of family means (based on error term from analysis of variance). These vary by family in the Horatio planting because of missing plots.

Table 2. Family means for plot volume, height and rust infection of select Carolina coastal families and checks planted near Stewart, Mississippi. They are ranked by height.

Family	Ht ft	Plot Volume Cu Ft	Rank	Rust Infection %	Rank
NC 8-61	35.0	124	(1)	10.9	(16)
NC 9-6	34.3	99	(9)	15.6	(19)
NC 8-131	34.1	109	(2)	11.7	(18)
SC 11-25	34.1	96	(12)	20.8	(24)
NC 8-136	34.1	76	(29)	13.2	(17)
NC 8-103	33.6	100	(7)	8.7	(10)
NC 8-73	32.9	104	(3)	10.0	(14)
SC 7-4	32.9	95	(14)	9.0	(12)
SC 11-16	32.7	94	(15)	2.0	(01)
SC 7-34	32.6	100	(5)	15.4	(19)
SC 7-58	32.4	86	(23)	37.0	(28)
NC 7-59	32.3	84	(25)	8.8	(09)
SC 11-10	32.2	84	(24)	6.8	(05)
SC F-39	32.1	87	(22)	15.8	(21)
AR + Tree	32.0	90	(19)	6.6	(04)
NC 8-27	32.0	88	(21)	16.0	(22)
SC 7-56	31.8	73	(30)	8.8	(11)
SC F-8	31.5	100	(6)	9.3	(13)
SC 18-34	31.1	104	(4)	11.7	(08)
NC 8-66	31.1	82	(27)	17.0	(20)
AR Check	31.0	99	(8)	3.2	(03)
MS-AL Ck	31.0	95	(13)	7.7	(07)
NC 7-88	31.0	92	(16)	19.0	(23)
NC 8-35	30.9	96	(11)	17.9	(20)
NC 9-4	30.8	91	(17)	8.5	(08)
SC F-67	30.7	98	(10)	7.2	(06)
NC Check	30.7	91	(18)	10.1	(15)
NC 8-46	30.4	89	(20)	24.2	(27)
NC 7-90	30.2	82	(26)	2.6	(02)
SC 11-21	28.9	77	(28)	23.2	(25)
SC 11-26	27.6	71	(31)	23.6	(26)
Mean	31.8	92		13.1	
Standard Error	1.1	8		2.8	

GROWTH AND YIELD OF GENETICALLY IMPROVED MATERIAL

MODERATED BY DR. RALPH C. BOWER

MacMillan Bloedel, LTD.

EFFECTS OF GENETICALLY IMPROVED STANDS ON GROWTH AND YIELD PRINCIPLES

P. T. Sprinz[1/]

Abstract.--Economic and biological evaluation of tree improvement programs is dependent on quantifying the long term inputs of improved selections on growth and yield response surfaces. Components and principles of growth and yield systems that may be affected by genetically improved stands are given. Specific examples of genotypic population effects on the height-age relationship and on the early dominant height-rotation yield relationship are presented. Suggestions are given for future research needs and direction.

Additional keywords: height-age, evaluation, loblolly pine, Pinus taeda.

The impact of tree improvement programs on forest management practices is considerable and seems to be increasing. Demand for genetically improved material has been based on the premise that the subsequent stands of selected trees will have better survival, growth rates, or quality characteristics, resulting in better yields and economic returns than stands of unimproved trees. However, present management decisions and future plans concerning the role of superior trees in an operational setting have been based largely on individual tree characteristics observed over the early years of selected research studies. Long-term studies covering a range of sites and silvicultural treatments, and containing large scale plantings of genotypic populations are not numerous, but they are critical for determining the dynamic growth and yield response surfaces and economic returns of genetically improved stands. These responses must be understood, quantified and integrated so that decision making systems can assess the true economic consequences of genetic improvement. An understanding of the effects of genetically improved stands on growth and yield is a necessary and logical starting point. My objective today is to discuss how genetically improved stands may affect growth and yield principles with specific reference to the height-age relationship.

GROWTH AND YIELD SYSTEMS

There are various components and principles of growth and yield models that can be affected by genetically improved material. The components of a growth and yield system will vary depending on the type of system. There are three basic systems: individual tree, diameter distribution and whole stand. An individual tree system has a tree as the basic modeling unit, while a diameter distribution system has a number of trees by diameter class as the modeling unit, and a whole tree system has a stand as the modeling unit.

1/ Assistant Professor, Department of Forest Science, Texas Agricultural Experiment Station, Texas A&M University System, College Station, Texas, 77843. The author wishes to express his appreciation to Weyerhaeuser Company and Texas Forest Service for their help in data collection.

The sophistication of a system, that is, the type of biological components and their interrelationships, and the level of output resolution, decreases from the individual tree model to the diameter distribution model to the whole stand model. For example, the individual tree model PTAEDA (Daniels and Burkhart 1975) for loblolly pine (Pinus taeda L.) contains the following functions describing biological components for the unmanaged portion of the program:

Pre-competition Phase	Post-competition Phase
Spatial distribution	
Point-of-competition	Competition index, crown ratio
Mortality	Probability of mortality
Height-age (site index)	Height-age (site index)
Diameter distribution	Diameter increment
Height, crown length	Height increment
	Volume, unit conversions

These components are not independent of each other. Although the parameters for each are estimated as if they are independent, they are actually interrelated in that an estimate from one function is often used in another function. Yield estimates are determined from the integration of these interrelationships. The output information in this system could include stand and stock tables for any specified age, and stand level statistics for individual tree characteristics of average diameter, height, crown length and competition index, and stand level characteristics of average basal area, yield and biomass. In addition, subprograms exist that can incorporate the effects of site preparation, fertilization and thinning.

In contrast, a whole stand model has only one biological component that incorporates all of the above individual tree components into one. For example, Burkhart et al. (1972) developed loblolly pine stand yield as a linear function of age, height of the dominants, and number of trees surviving. This function requires essentially the same input as the individual tree system, but the whole stand model supplies only one piece of information - an aggregate yield for a specified input.

The effects of genetic improvement may be found in any or all of the components of any of the yield systems. Since geneticists select genotypes on the basis of survival (e.g., drought hardy), growth rates (dominant height), productivity (specific gravity) and quality (disease resistance) characteristics, it would seem that the growth and yield components characterized by these selection criteria would be naturally affected. However, the growth and yield components, as in the case of an individual tree model, are interrelated meaning that if one component is affected by genetic selections, then most of the remaining components will be affected by that single change as well as any final yield estimates. For example, in the case of PTAEDA, if the site index or height-age function is adjusted for differences in genotypic populations, there will be associated changes in survival, tree size attributes, distributions, growth responses, and taper. In the end, the proposed genetic adjustment in height-age may not result in the desired adjustment in total yield because of the resulting interactions (Sprinz 1986).

There are some important growth and yield principles that may be also affected by genetically improved stands. Langsaeter's growth-growing stock (1944, taken from Smith 1986), maximum stocking line (Reineke 1933, Drew and Flewelling 1977) and carrying capacity are other biological relationships that have been hypothesized for testing to determine if significant effects from tree improvement exist (Nance et al. 1986). Information from analyzing genetic effects on these principles will have important ramifications on how growth and yield systems perform as an aggregate. In the example above, a proposed change in site index due to genetic differences would affect these principles as well as the components of growth and yield systems.

GENETIC EFFECTS ON HEIGHT-AGE

With this idea of growth and yield as a system of interrelated components, I would like to present some results from analyzing the effects of different genotypic populations on one component - the height-age relationship. Height has been an important trait that geneticists have used in selecting superior genotypes and in determining potential gains at later dates. It has also been one of the methods discussed as a way to incorporate the effects of tree improvement into growth and yield modeling.

Most of the reported height-age work has centered on quantifying effects of genetic populations on the shape and level of this relationship, and on determining subsequent effects of changes in this relationship on aggregate yields. Nance and Wells (1981b) and Buford and Burkhart (1985) have analyzed loblolly pine genotypic population effects on the shape and level attributes of a logarithm of height and inverse of age model. They found significant differences in the level of the height-age relationship by population. This result would indicate that adjustments in site index values may be a valid method in adjusting growth and yield models for differences in genetic populations. In other research, Schmidtling (1984) found shape and level differences between loblolly families selected for high and low specific gravity using the same height-age model.

Few studies exist that have repeated measurements of height over time corresponding to actual yields for the entire length of a rotation. Through a growth and yield simulation of loblolly pine, Nance and Bey (1979) determined that in general, height gains that diminished over the length of the rotation (i.e., shape differences) had minimal effect on final yields; however, a consistent height gain over the length of a rotation (level differences) had a considerable impact on final yields. Diminishing height gains can be thought of as temporary lift in site index, while consistent height gains can be thought of as a permanent lift in site index (Fig. 1). Nance and Wells (1981a) incorporated dominant height differences in a growth and yield model and found greater source differences in yield than were actually observed. Taking an early percent height gain as a percent site index gain, Talbert (1982) simulated final yields and determined that yields were tending to converge by age 35. Wells (1983) used dominant height differences at age 25 to determine potential yield differences. He found that estimated yields at age 25 of seed sources grown in Arkansas were generally not different.

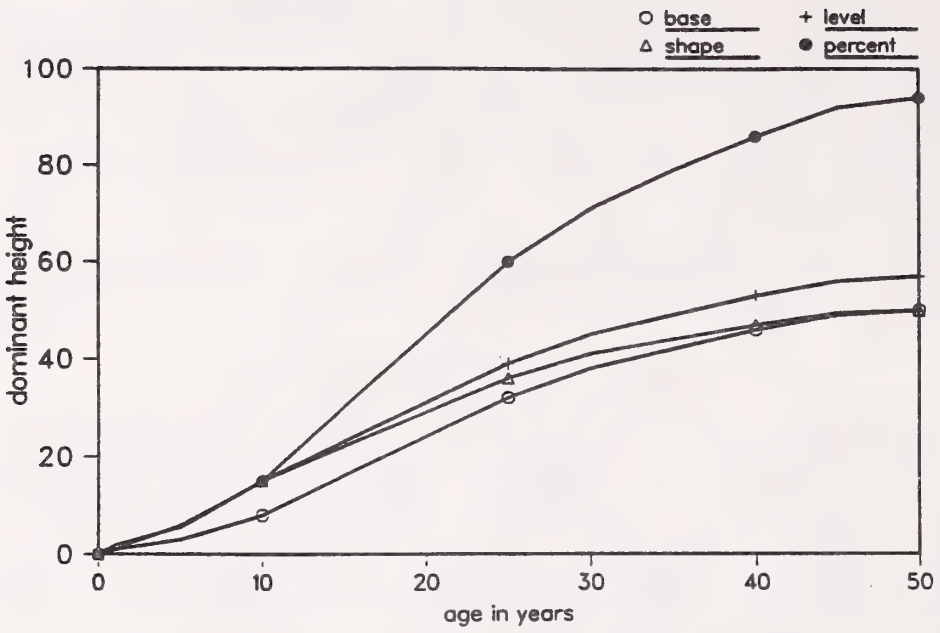


Figure 1. Dominant height-age trends showing level, shape and percent differences of an assumed height increment at age 10.

STUDY AREAS

Texas A&M University and Weyerhaeuser Company have worked closely over the last two years collecting information from several loblolly pine genetic trials. One study was the Southern Pine Seed Source Study (SPSSS) located in southwestern Arkansas, while the others were Texas Forest Service half-sib block plantings located in western Louisiana.

The particular portion of the SPSSS that we sampled consists of four spatially separated completely randomized blocks of 36 geographic seed sources representing the native range of loblolly pine. Historical data on diameter at breast height, total height and survival were available (Grigsby 1973, Grigsby 1977, Wells and Lambeth 1983) through age 26 from seed. Wells and Lambeth (1983) from their analyses subdivided the sources into 7 geographic regions (Fig. 2). At age 29 from seed, the study was measured for diameters and heights, with a destructive subsample taken for stem analysis. This procedure entails cutting a tree into 5-ft sections and then splitting the sections to determine the exact height at each age through flush or whorl counts. The height and age pairs were recorded on 279 trees.

The Texas Forest Service studies consist of completely randomized blocks of selected genotypes. One study contains 7 superior half-sib families planted adjacent to corresponding check families. The block plantings were either 31 or 34 years from seed when 56 trees were stem analyzed for height-age pairs. Another study contained 20 block plantings of families selected for high or low specific gravity. In this case there were 48 trees sampled at age 31 years from seed.

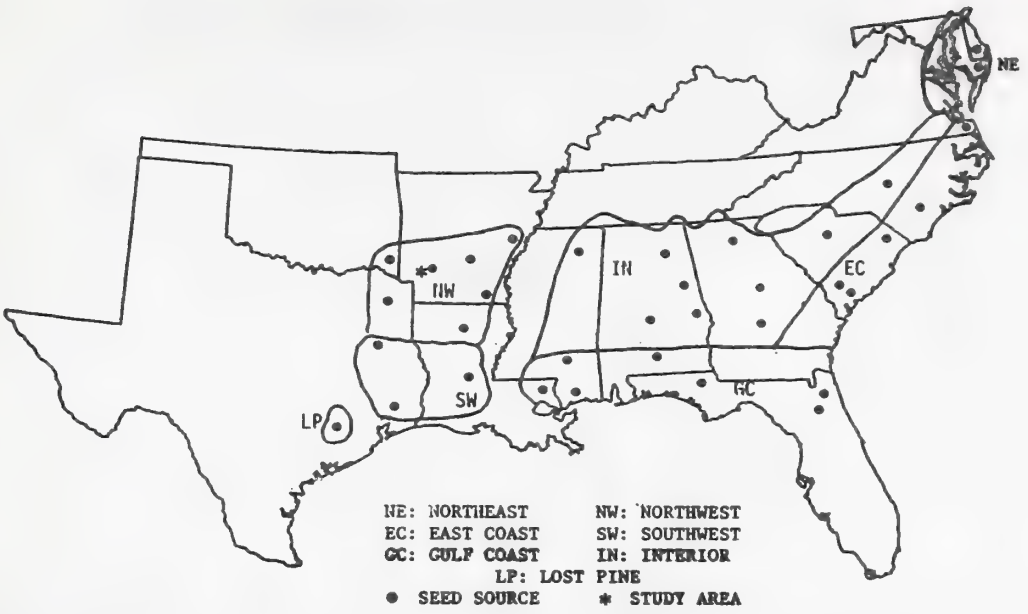


Figure 2. Map indicating regional groupings, seed source groupings and study area.

METHODS OF ANALYSIS

There were two main methods used to evaluate genetic effects on the height-age trends. One method involved testing significant differences among average values and specific contrasts with an analysis of variance procedure.

The second method entailed testing significant differences among parameters of a height-age model with a full and reduced model procedure. Two models were tested. A logarithm height and inverse of age model was initially tested, but proved to be inappropriate in describing the sigmoidal curve exiting between height and age (Fig. 3) (Sprinz 1986). The other model, popularized by Pienaar and Turnbull (1973), proved to be very flexible and suitable for this analysis (Fig. 3). This model is characterized as

$$H = a(1 - \exp(-b*A))^c \qquad (1)$$

where,

H = total height (ft) based on stem analysis
A = age from seed (years)
exp = exponential
a,b,c = regression coefficients.

The parameters of this model have important interpretations that may be useful in describing the effects of genetics on the height-age relationship. The asymptote of the curve is describe by the 'a' parameter, the rate of development is indicated by the 'b' parameter, while the pattern of development is measured by the 'c' parameter. Namkoong et al. (1972) found that the parameters of model (1) were not significantly affected by Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) populations even though other analyses indicated significant population differences in height.

Figure 3. Observed dominant height-age trend compared to predicted values from a logarithm of height and inverse of age model, and a nonlinear model (1), proposed by Pienaar and Turnbull (1973).

Significant differences between these parameters will give an indication of where genetically improved stands are affecting the height-age relationship and how these effects can be incorporated into growth and yield models. One method to determine significant differences is to compare the residual mean square associated with models fit to each region separately (i.e., full model) to the residual mean square associated with models combining regions (reduced model) (Neter et al. 1983). For example, a model containing one asymptote parameter and region specific rate and pattern parameters may have a similar residual mean square as a model containing region specific asymptote, rate and pattern parameters. In this example, the inference would be that genetic gains were found in the shape of the height-age trend, but not the level.

RESULTS AND DISCUSSION

Average total height and dominant height in the seed source trials were consistently and significantly taller for eastern versus western sources, and for East Coast versus Northwest sources over the 29-year period.[a] Dominant heights for the eastern sources were 1.3 ft (4.7%) taller than the western sources at age 11, and 4.5 ft (6.7%) taller at age 29 (Fig. 4). The East Coast sources were 2.5 ft (9.3%) taller than the Northwest sources at age 11 and were 5.2 ft (7.7%) taller by age 29 (Fig. 5). These differences translated in a 4.0 ft increase in site index at base age 25 for the eastern sources compared to the western sources, and 5.1 ft increase for the East Coast versus Northwest sources.

Results from Model (1) suggested that the general shape of the height-age trend was affected by the geographic populations. It was found that a common asymptote parameter with source related rate and pattern parameters could be used across the regions. This would suggest that the maximum dominant and codominant heights among seed sources are similar; however, the height-age

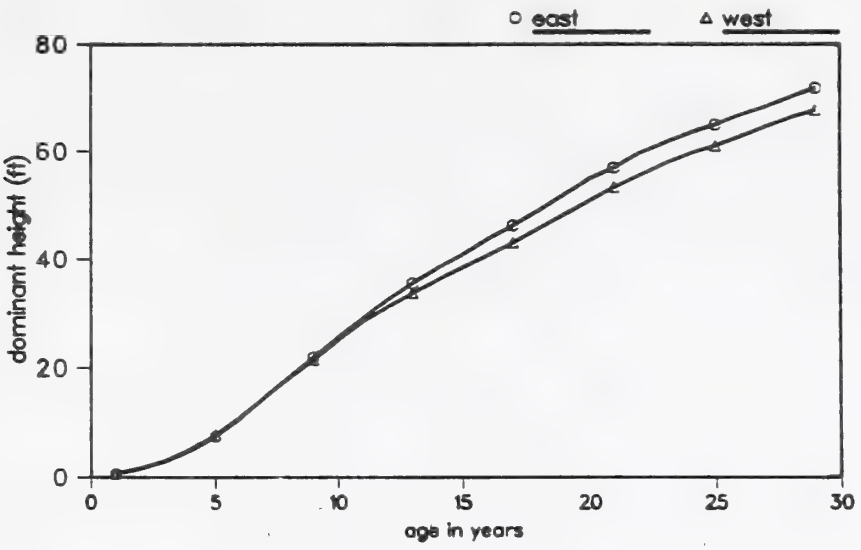


Figure 4. Observed dominant height-age trends from western and eastern loblolly pine seed sources grown in Arkansas.

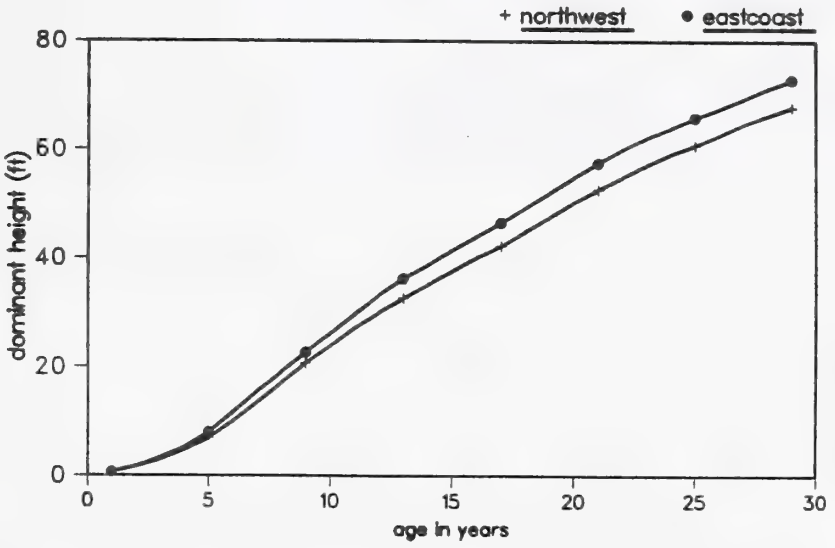


Figure 5. Observed dominant height-age trends from the Northwest and East Coast loblolly pine seed sources grown in Arkansas.

relationship prior to the maximum is different. Nance and Wells (1981b) and Buford and Burkhart (1985) found that level rather than shape differences existed when using a logarithm of height and inverse of age model.

The block plantings of superior versus check, and low versus high specific gravity indicated different results than found from the seed source trials. In the case of superior and check plantings, specific contrasts suggested that in 2 cases there were not significant difference in any parameters, in 2 cases were only different asymptotes (superior asymptotes at a higher level), and in 3 cases there were only differences in rate or pattern (2 of the 3 superior families developed taller until base age).

In the case of low versus high specific gravity plantings, it was indicated that a 1.7 ft (4.9%) advantage for the low gravity material at age 11 from seed resulted in a 4.3 ft (6.4%) gain by age 26 from seed. In addition, only asymptote differences existed in model (1) - the low specific gravity plantings having the higher asymptote than the high specific gravity material (Fig. 6). Schmidtling (1984) using a logarithm height and inverse of age model found both level and shape differences between families selected for low and high specific gravity: low specific gravity families were taller than high specific gravity families to age 21 at which point the high gravity families became taller than the low gravity families.

Problems also exist if early attribute data from plantings are used to make long term projections of final aggregate yields. If early dominant height differences from these studies are related to actual yields at rotation age, trends were not obvious. Within each study, the height gains indicated at age 11 from seed were regressed with the final standing yields. An absolute or percentage gain in height was not related to final yield in any of the studies as shown in Figure 7 with the seed source trials and Figure 8 with the superior-check family plantings.

---

[a] Sprinz, P. T., C. B. Talbert, and M. R. Strub. Height-age trends from an Arkansas seed source study. Submitted to Forest Science.

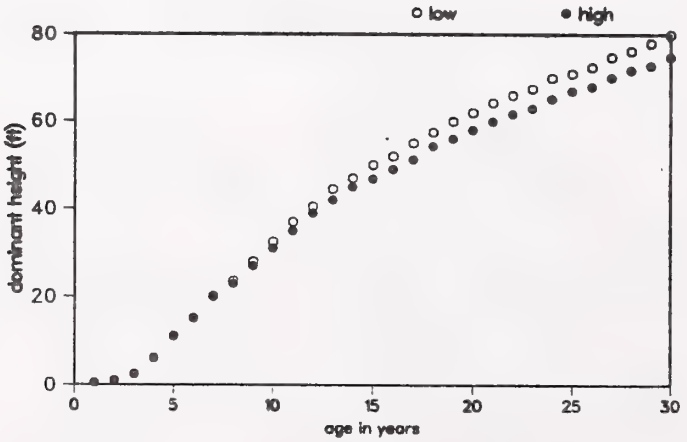


Figure 6. Observed dominant height-age trend from half-sib block plantings of families selected for low and high specific gravity.

SUMMARY

These results were given to demonstrate the complexity of determining the effects of genetic improvement on one component of a growth and yield system. As a mixture of results from across widely varying genotypic populations these may be disconcerting. Within a study, however, the findings are very meaningful and are helpful in understanding the genetic effects on the dynamic response surfaces found in growth and yield. For example, the results from the seed source trials provided the type of data we needed to understand the long term stand dynamics of and tree relationships in genotypic populations. In the case of the specific gravity contrasts, we are investigating the different growth strategies exhibited in the differential development of height.

At Texas A&M we are investigating the genetic effects on other growth and yield components and principles. These areas include height-diameter, diameter distributions, taper, volume, quality, growth-growing stock and carrying capacity. Results have not always been clear and cohesive, especially as we look across a wide spread of genotypic populations; however, it is a start to understanding, quantifying and incorporating genetic improvement into growth and yield.

The greatest handicap that the tree improvement program has is the time factor. A 30-year period is not an acceptable period for evaluating a cross; however, evaluating row plots at age 10 without an understanding of intraspecific competition effects on stand attributes is also not acceptable. Studies exist now and designed studies can be established that will enable scientists to quantify population dynamics of genetically improved stands, which in turn will provide a sounder basis for evaluating early selections and for projecting rotation gains. This research will have to transcend disciplines if the correct hypotheses are to be developed and correct inferences to be made. Evaluation of the biological and economical aspects of genetic improvement necessitates a systems approach if there will be success in this endeavor. Only a systems approach can integrate the knowledge of the geneticists, biometricians, silviculturists, economists and land managers in assessing tree improvement.

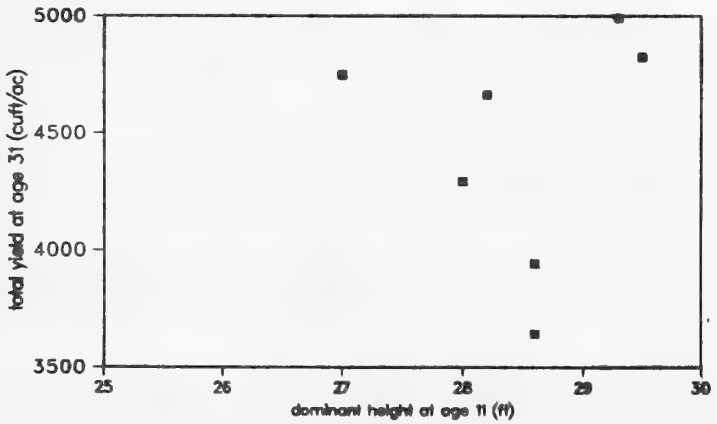


Figure 7. Comparisons of standing yield at age 31 and dominant height at age 11 of loblolly pine seed sources grown in Arkansas.

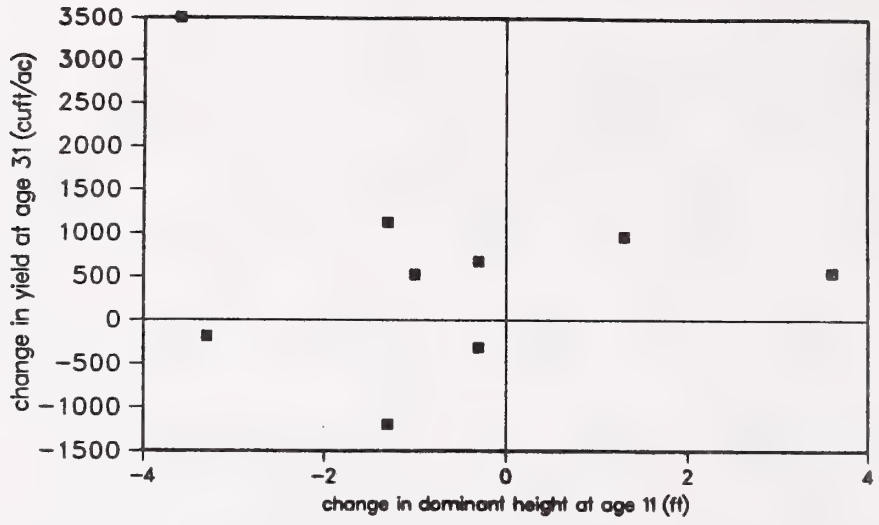


Figure 8. Incremental gain of superior over check half-sib families in terms of standing yields at age 31 and dominant height at age 11.

LITERATURE CITED

Buford, M. A., and H. E. Burkhart. 1985. Dynamics of improved loblolly pine plantations and the implications for modeling growth of improved stands. P. 170-178 in Proc. 18th South. Forest Tree Improv. Conf., Gulfport, MS.

Burkhart, H. E., R. C. Parker, M. R. Strub, and R. G. Oderwald. 1972. Yields of old-field loblolly pine plantations. Va. Polytech. Inst. and State Univ., Div. For. Wildl. Resour., Pub. No. FWS-3-72, 51 p.

Daniels, R. F., and H. E. Burkhart. 1975. Simulation of individual tree growth and stand development in managed loblolly pine plantations. Va. Polytech. Inst. and State Univ., Div. For. Wildl. Resour., Pub. No. FWS-5-75, 69 p.

Drew, T. J., and J. W. Flewelling. 1977. Some recent Japanese theories of yield-density relationships and their application to Monterey pine plantations. Forest Sci. 23:517-534.

Grigsby, H. C. 1973. South Carolina best of 36 loblolly pine seed sources for southern Arkansas. USDA Forest Serv. Res. Pap. SO-89, 10 p.

Grigsby, H. C. 1977. A 16 year provenance test of loblolly pine in southern Arkansas. P. 261-268 in Proc. 14th South. Forest Tree Improv. Conf., Gainesville, FL.

Namkoong, G., R. A. Usanis, and R. R. Silen. 1972. Age-related variation in genetic control of height growth in Douglas-fir. Theor. Appl. Genetics 42:151-159.

Nance, W. L., and C. F. Bey. 1979. Incorporating genetic information in growth and yield models. P. 140-148 in Proc. 15th South. Forest Tree Improv. Conf., Starkville, MS.

Nance, W. L., and O. O. Wells. 1981a. Estimating volume potential in genetic tests using growth and yield models. P. 39-46 in Proc. 16th South. Forest Tree Improv. Conf., Blacksburg, VA.

Nance, W. L., and O. O. Wells. 1981b. Site index models for height growth of planted loblolly pine (Pinus taeda L.) seed sources. P. 86-89 in Proc. 16th South. Forest Tree Improv. Conf., Blacksburg, VA.

Nance, W. L., B. G. McCutchen, C. B. Talbert, M. A. Buford, C. S. Foster, and P. T. Sprinz. 1986. Experimental approaches for evaluating genetic effects on stand growth and yield. P. 22-39 in Statistical Considerations in Genetic Testing of Forest Trees, the Proc. of the 1986 Workshop of South. Regional Informtion Exchange Group 40. Inst. of Food and Agricultureal Sciences, Univ. Florida, South. Coop. Series Bulletin No. 324.

Neter, J., W. Wasserman, and M. H. Kutner. 1983. Applied Linear Regression Models. Richard D. Irwin, Inc., Homewood, IL.

Pienaar, L. V., and K. J. Turnbull. 1973. The Chapman-Richards generalization of von Bertalanffy's growth model for basal area growth and yield in even-aged stands. Forest Sci. 19:2-22.

Reineke, L. H. 1933. Perfecting a stand-density index for even-aged forests. J. Agr. Res. 46:627-638.

Schmidtling, R. C. 1984. Early intensive culture affects long-term growth of loblolly pine trees. Forest Sci. 30:491-498.

Smith, D. M. 1986. The Practice of Silviculture. 8th edition, John Wiley and Sons, New York. 527 pp.

Sprinz, P. T. 1986. Growth and yield relationships for genetically improved stands. P. 80-84 in Proc. of the 1986 Society of Amer. For. Nat. Convention. Birmingham, AL.

Talbert, J. T. 1982. One generation of loblolly pine tree improvement-results and challenges. P. 106-120 in Proc. 18th Canadian Tree Improv. Assoc. Mtg., Duncan, B.C.

Wells, O. O. 1983. Southwide pine seed source study - loblolly pine at 25 years. South. J. Appl. For. 7(2):63-71.

Wells, O. O., and C. C. Lambeth. 1983. Loblolly pine provenance test in southern Arkansas: 25th year results. South. J. Appl. For. 7(2):71-75.

PATTERNS OF GENETIC VARIATION AMONG TEN-YEAR-OLD OPEN-POLLINATED MID-SOUTH SEED SOURCES OF AMERICAN SYCAMORE[1]

J. R. Toliver and S. G. Dicke[2]

Abstract.--One-year-old seedlings of 96 open-pollinated American sycamore (Platanus occidentalis L.) families from 16 seed sources in AL, MS, LA, and AR were planted in a compact family design in south-central LA in 1976. Seed source and family components of genetic variation were significant for height, dbh, volume, and stem canker infection, and would allow for substantial gains through source and family-within-source selection. The best 9 families (top 10%) came from the two Mississippi river sources closest to the plantation. The means for these families in this plantation were better than the plantation mean by 2.1 m for ht, 2.4 cm for dbh, 26.6 cu decimeters for volume, 14% for survival, and 29% less for stem canker infection at age 10 years.

Additional keywords: geographic variation, progeny testing.

INTRODUCTION

American sycamore is a common bottomland hardwood widely distributed throughout the eastern half of the United States. Until recently its commercial value was limited to package veneer, factory and box lumber, and furniture (Putnam et al 1960). However in the early 1970's sycamore was proposed as an excellent species for short- rotation production of biomass for energy and paper pulp (Steinbeck et al 1972). Research has included investigation of genetic variation available to increase growth and yield in plantations.

In Louisiana, sycamore is one of several hardwood species planted to convert old bottomland fields to commercially productive forests. Such conversion has recently increased, resulting in a demand for genetically improved sycamore seedlings. This study provides information needed to implement a program of selection which should result in an increase in volume production of sycamore at a time of increasing demand for hardwood fiber in Louisiana and neighboring states.

---

[1]Paper presented at the 19th South. For. Tree Impr. Conf., College Station, Texas, June 16-18, 1987.

[2]Associate Professor and Instructor, respectively, School of Forestry, Wildlife, and Fisheries, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA.

MATERIALS AND METHODS

Site Description

Location - The study was established on the Thistlethwaite Wildlife Management Area, four miles northeast of Washington, LA (latitude 30°40' N, longitude 92°0' W) (Figure 1), in the Red River Region of the Lower Mississippi River Delta.

Soil - The soil is a Dundee silty clay loam, a member of the fine-silty, mixed, thermic family of Aeric Ochraqualfs (Murphy et al 1986). It is a level, somewhat poorly drained, and medium in fertility alluvial soil located just off the bank of Bayou Wauksha. Having been in cultivation for over 20 years, the soil is moderately compacted, and contains a plowpan at a depth of about 25 cm.

Based on the species composition of surrounding timber stands, the site is best suited for cherrybark oak (Quercus falcata var. pagodifolia Ell.) and sweetgum (Liquidambar styraciflua L.). According to Broadfoot (1976) the site index of sycamore for a Dundee silty clay loam ranges from 29 to 35 m in 50 years.

Figure 1. Location of seed sources and planting test site used to evaluate variation of ten-year-old American sycamore characteristics.

Plantation Description

Genetic Material - Sixteen seed sources from the Mid-South region (Figure 1) were tested at this site as part of a cooperative study. Dr. S. B. Land, Jr., Mississippi State University, provided the genetic material. Table 1 contains a description of each seed source location. Seed was collected from two stands at each seed source and from three open-pollinated parent trees per stand (six trees per seed source) making a total of 96 families tested. No selection criteria were used during seed collection. Seedlings were grown at the Winona Nursery of the Mississippi Forestry Commission.

Experimental Design - The plantation was laid out as a compact family design, a split-plot within a randomized complete block design. Seed sources were blocked within each of six replications, and three-tree row plots for each family were randomly placed within their source block.

Establishment and Maintenance - In March, 1976, 1-0 seedlings were planted on 3m x 3m spacing using KBC bars. No site preparation was necessary as the seedlings were planted on soybean stubble. Weed control consisted of discing between trees twice each year during the first two years and mowing twice each year during the next two years. No thinning was applied in the plantation.

Table 1. Location of seed sources used to study genetic variation of ten-year-old American sycamore characteristics.

Source Code	State	County/ Parish	River Drainage	Lat. (North)	Long. (West)
S	AR	Grant and Saline	Saline	34°25'	98°38'
H	AR and MS	Phillips and Coahoma	Mississippi	34°27'	90°40'
T	MS	Marshall	Tallahatchie	34°35'	89°29'
I	MS	Itawamba	Tombigbee	34°22'	88°26'
C	AR	Drew	Saline	33°28'	91°54'
G	MS	Bolivar	Mississippi	33°37'	91°09'
K	MS	Attala	Big Black	33°34'	89°58'
L	AL	Pickens	Tombigbee	33°16'	88°18'
O	LA	Catahoula	Ouachita	31°52'	91°54'
N	MS	Jefferson	Mississippi	31°47'	91°15'
J	MS	Copiah	Pearl	32°26'	90°15'
A	AL	Choctaw and Marengo	Tombigbee	32°15'	87°28'
F	LA	Pt. Coupee and W. Feliciana	Mississippi	30°53'	91°38'
B	LA and MS	Washington and Pearl River	Pearl	30°46'	89°50'
P	MS	George	Pascagoula	30°48'	88°43'
M	AL	Baldwin	Tombigbee	30°57'	87°55'

Characteristics Studied

Tenth-year-measurements were taken during February - March,1986, and included diameter at breast height (0.1 inches) and total height (ft). A visual estimate was made on the percentage of stem length that had been killed by sycamore stem canker. This stem disease was believed to be caused primarily by two canker fungi, Ceratocystis fimbriata Ellis and Halst. and Botryodiplodia theobromae Pat. (Cooper et al 1977). Stem volume inside bark (i.b.) was calculated using the following equation from Land (1982):

$$\text{Volume} = 2.26 + .0335 \times (\text{dbh}^2 \times \text{ht})$$

where volume = stem volume i.b. in cubic decimeters (cu dm); dbh = diameter at breast height in cm; and ht = total height in m.

Statistical Analysis

Tests of significance for sources of variation were done on a plot mean basis using the glm procedure of Statistical Analysis System (SAS Institute, Inc. 1985). All sources of variation were assumed to be random. Influence of seed source latitude on sycamore characteristics was evaluated by separating seed source variation into variation among the four latitudinal transects (Figure 1) and variation within the transects. When F-tests were significant Duncan's multiple range test, (alpha = .05) was used to separate means. Genetic variance estimates were calculated using mean squares from analysis of variance.

RESULTS AND DISCUSSION

Plantation Averages

Height at age 10 yrs averaged 9.56 m. Average dbh was 10.4 cm and, stem volume inside bark averaged 45 cu dm. Survival was 80% at age 10 and 30% of the plantation was infected by stem canker fungi. Stem cankers killed an average of 70% of the height and 48% of volume i.b. on infected trees. Half of the trees infected by stem canker were dead at age 10. Death from stem cankers accounted for 72% of the total mortality in the plantation during the first 10 years. Thus, survival was heavily dependent on stem canker resistance and additional mortality will most likely occur as Land (1982) found that most trees die within two years of stem canker formation. Smaller, weaker trees in this plantation had the greatest stem canker infection. No disease was found on the largest trees, 15 to 19 cm in dbh. To reduce the influence of cankers, only heights and volumes of healthy trees were used and dbh was used for analysis only when trees had 20 percent or more of their stem length alive.

Analysis of Variance

A ranking of the genetic components indicates that seed source variation was greater than family variation, which was greater than stand variation (Table 2). With the exception of survival, stand variation within seed source was low and considered to be unimportant. Stand variance for survival was significant but there was no family variance. Environmental variation (rep) and G x E variation (rep x seed source) were significant for height, dbh and volume. Only genetic components of variation were significant for stem canker infection supporting the conclusion that all trees have been exposed to the disease.

Table 2. Estimated values and significance levels of variance components (σ^2) in a ten-year-old American sycamore progeny test near Washington, LA.

Source of Variation	Characteristic at age 10 years				
	Height (m)	DBH (cm)	Volume i.b. (cu dm)	Survival (%)	Stem Canker Infection(%)
---------- Genetic variation estimates (σ^2) ----------					
Seed Source (P)	.984**	.881**	95.0**	220.7**	715.3**
Latitude(L)	.299	.162	0	252.8**	732.1**
Source/L	.681**	.661**	89.8**	21.1	66.6*
Stand (S)/P	0	.097	8.0	31.9*	1.2
Family (F)/SP	.117**	.331**	20.3**	0	82.1**
---------- Environmental and G x E variation estimates (σ^2) ----------					
Rep (R)	.401**	.496**	36.3**	8.1	16.0
P x R	.659**	.877**	71.2**	22.6	31.8
L x R	.182*	.077	17.8	20.9*	3.0
S/L x R	.504**	.839**	55.0**	3.6	29.5
S/P x R	.019	.198	20.0	22.6	1.6
Error	1.011	2.636	195.7	429.4	504.7

** = significant at the .01 level of probability
* = significant at the .05 level of probability

Seed Source Variation

Latitude of seed source explained most of the variation in percent infection by stem cankers (Table 2). Southern sources were more resistant to infection while sources from the northern most latitude were highly susceptible. Forty to 80% of Arkansas and northern Mississippi source trees (H,I,S,T) were infected by stem canker by age 10 (Table 3). The same trend was found by Land (1982) on a test site at Vicksburg, MS, containing the same seed sources. In both studies, resistance to stem canker infection was

found in sources from southeastern LA and along the lower portion of the Mississippi river. The best two seed sources at age 10 were F (West Feliciana Parish, LA) and N (Jefferson County, MS) for height, dbh, and volume (Table 3). They were also lowest in percent of trees with stem canker infection. Both sources were located along the Mississippi River and were the closest to the test site. They were 1.8 m (19%) taller than the plantation average in height and 2.0 cm (20%) larger in dbh. These two sources on average produced 22 cu dm (49%) more volume per tree, had 16% better survival and had 29% less stem canker infection. Seed sources F and N were also the best in growth and stem canker resistance when tested at a site near Vicksburg, MS (Land 1982). Similar performance of sources at the two sites indicates that the source G x E interaction is not very strong.

Table 3. Seed source means for American sycamore characteristics in a ten-year-old progeny test near Washington, LA.

Seed Source	Characteristic at age 10 years [1/]				
	Height (m)	DBH (cm)	Volume ib (cu dm)	Survival (%)	Stem Canker Infection(%)
N	11.51a	12.35a	66.2a	90.7ab	0.9a
F	11.24ab	12.45a	67.8a	97.2a	0.0a
B	10.84b	11.25b	53.0bc	89.8ab	1.9s
G	10.10c	11.38b	54.6b	87.2ab	21.2cd
A	9.94cd	10.56bc	45.7cd	92.6a	27.8de
0	9.74cde	11.36b	49.3b	92.6a	5.2ab
K	9.43def	9.94cde	39.4def	89.2ab	23.7d
P	9.40def	9.98cde	38.6def	92.5a	16.1bcd
J	9.32ef	10.40cd	41.0de	93.9a	3.5a
T	8.94fg	9.29ef	36.7ef	65.8c	71.9i
M	8.93fg	9.53def	35.3ef	89.2ab	11.8ac
C	8.47gh	9.39ef	33.6ef	80.6b	44.4f
H	8.35hi	9.89cde	37.9def	64.8c	55.7h
L	8.28hi	9.26ef	33.6ef	79.6b	35.2ef
S	7.85i	8.95fg	31.0f	41.7d	80.6i
I	6.41j	8.22g	21.0g	42.5d	80.0i
Plantation Average	9.56	10.36	45.0	80.6	29.9

[1/] Seed source means followed by different letters within a characteristic were significantly different at alpha = .05 (Duncans multiple range test).

Family Variation

Family means at age 10 ranged from 4.6 to 12.0 m for height; 5.2 to 14.6 cm for dbh; 17 to 92 cu dm for volume i.b. per tree; and 8 to 100% for survival. Of the 96 families 12 had 100% survival. No canker infections were found in 28 families while 3 families from seed source I, Itawamba County, MS, had 100% infection. When the above 28 disease-free families

were tested at Vicksburg, MS (Land 1982) 12 had some trees with stem cankers. In addition, 10 disease-free families at Vicksburg had some diseased trees in this study. This suggests there may be a strong family G x E interaction in sycamore resistance and/or pathogen virility relative to plantation location.

The best nine families (top 10%) in height (Table 4) came from the best two seed sources, F and N. The mean performance of these nine families was greater than the plantation average by 2.1 m in height, 2.4 cm in dbh, 6.6 cu dm in volume i.b. per tree, and 14% in survival. In addition, these families had 29% less stem canker infection.

Table 4. Characteristic values of the best 9 families (top 10%) in a ten-year-old American sycamore progeny test in south-central LA.

Family Description			Characteristic at age 10 years				
Source	Stand	Family	Height (m)	DBH (cm)	Vol. i.b. (cu dm)	Survival (%)	Stem Canker Infect.(%)
F	1	1	11.96	14.55	91.86	100.0	0
F	1	8	11.76	13.26	76.25	100.0	0
N	2	4	11.71	13.47	78.07	88.9	5.6
F	2	4	11.67	12.76	72.73	83.3	0
N	1	10	11.63	12.29	66.52	100.0	0
N	2	8	11.56	11.88	59.85	94.4	0
F	2	1	11.55	11.98	65.07	100.0	0
N	2	1	11.44	12.83	70.16	94.4	0
N	1	5	11.48	12.14	64.28	94.4	0
Top Families Average			11.64	12.80	71.64	95.1	.6
Plantation Average			9.56	10.36	45.02	80.6	29.9
Difference			2.08	2.44	26.62	14.5	-29.3

CONCLUSIONS

Source of seed for seedlings can be very important when planting sycamore in Louisiana, particularly relative to stem canker disease resistance. Substantial gains in survival, height, diameter, volume, and stem canker resistance can be obtained in south-central Louisiana if sycamore seed is collected from sources in the lower Mississippi river valley (Natchez, MS and south). Further gains can be attained if family-within- source selection is performed.

LITERATURE CITED

Broadfoot, W. M. 1976. Hardwood suitability for and properties of important Midsouth soils. South. For. Exp. Stn., New Orleans, LA. U.S.D.A. For. Serv. Res. Pap. SO-127. 84p.

Cooper, D. T., T. H. Filer, Jr., and O. O. Wells. 1977. Geographic variation in disease susceptibility of American sycamore. South. J. of Applied For. 1(4):21-24.

Land, S. B., Jr. 1982. Genetic selection of American sycamore for biomass production in the Mid-South. Final report for subcontract 9051 of U.S. Dept. of Energy Contract W-7405-eng-26. U.S. Dept. ov Commerce, National Technical Information Service, Springfield, VA. 114p.

Putnam, J. A., G. M. Furnival, and J. S. McKnight. 1960. Management and inventory of southern hardwoods. U.S.D.A. For. Serv. Agric. Handbk. 181. 102p.

Murphy, K. E., J. K. Vidrine, and D. R. McDaniel. 1986. Soil survey of St. Landry Parish, Louisiana. U.S.D.A. Soil Conservation Service. 192p.

SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC: SAS Institute Inc., 956p.

Steinbeck, K., R. G. McAlpine, and J. T. May. 1972. Short rotation culture of sycamore: a status report. J. For. 70:210-213.

GEOGRAPHIC GENETIC VARIATION IN BLACK CHERRY

C. S. P. Patil[1/] and S. B. Land, Jr.[2/]

Abstract.--Geographic genetic variation for survival, tree growth, and black knot disease susceptibility in a five-year-old black cherry provenance test was examined for relationships with three geographic and eleven climatic variables. Tree growth varied clinally; sources from near the Gulf and Atlantic coasts grew faster than interior sources when planted in central Mississippi. Annual cooling degree days and the amount of precipitation during the summer provided the best explanation of the observed patterns of geographic genetic variation. Seven geographic subregions of closely related provenances with distinct characteristics were identified.

Additional keywords: Prunus serotina, provenance test.

A certain amount of risk is involved in any tree improvement program if it is initiated without prior information on geographic genetic variation. The importance of such information has already been recognized in many forest tree species. Some information on geographic variation in black cherry (Prunus serotina Ehrh.) is available (Barnett 1977, Cech and Kitzmiller 1968, Cech and Carter 1979, Earnst 1978, Genys and Cech 1975, Wright and Lemmien 1974) but these earlier studies lacked coverage over much of the southern portion of the range. The objective of the present study is to examine and describe the geographic genetic variation of black cherry throughout its range.

MATERIALS AND METHODS

During 1979 and 1980 an extensive collection of open-pollinated seeds from 46 locations across 20 states in the Eastern United States was made by the U. S. Forest Service (Figure 1). The procedures followed for seed collection, seed extraction, and seedling production in the nursery have been described by Pitcher (1984).

In 1982, one-year-old seedlings were outplanted at the Truck Crops Branch Experiment Station of the Mississippi Agricultural and Forestry Experiment Station (MAFES) located near Crystal Springs, Mississippi. Ninety open-pollinated families representing 35 seed sources were planted in a randomized complete block design having four replications and four-tree family row plots. The family plots are not grouped by sources, but rather

---

1/Visiting Scientist from India under USAID program, Department of Forestry, Mississippi State University, Mississippi State, MS 39762 (Associate Professor, UAS, Dharwad-580005, India).

2/Professor, Department of Forestry, Mississippi State University, Mississippi State, MS 39762. Contribution No. 6698 of the Mississippi Agricultural and Forestry Experiment Station.

Figure 1. Locations of seed sources in the black cherry range.

are randomly assigned within replications. Spacing among trees is 10 feet by 10 feet, and the planting is surrounded by two border rows.

Survival, dbh, tree height, and black knot disease infection (Dibotryon morbosum) were measured five years after the study was planted. These four traits are hereafter called the dependent variables. When a tree was alive and less than 4.5 feet in height, then a value of zero was assigned for dbh. Three geographic characteristics of each provenance--latitude, longitude, and elevation--and eleven climatic traits of each provenance--mean annual temperature (MANTEMP), mean maximum (MAXTEMP) and minimum (MINTEMP) temperatures, mean annual precipitation (MANPREC), the sum of the mean monthly precipitation for June, July and August (SMOPREC), annual heating degree days (ANHTDD) and cooling degree days (ANCLDD), mean number of frost-free days (above 32°F) (MFRFRD), number of days after December 31 to the last 32°F temperature in the spring (MDTOL32), precipitation for the 100 days after the mean date of the last 32°F in the spring (PREC100), and sum of growing degree days above 42°F for the first 100 days after last frost (SGROWDD)--were obtained by the U. S. Forest Service personnel from the National Climatic Center in Asheville, NC. These are referred to as the independent (or predictor) variables in the analyses of this study.

SAS procedures (SAS Institute 1985) were used for all analyses. Analyses of variance, using SAS procedure PROC GLM, were conducted on plot means for each of the dependent variables to test for significance of provenance variation and family-within-provenance variation. Canonical correlation procedure (PROC CANCORR) was used to obtain preliminary information on the relationships between dependent and predictor variables. Principal component analysis was carried out on source means by PROC PRINCOMP to get a weighted combination of the four traits. The standardized first principal component was then used as the dependent variable in

stepwise multiple regression analysis (PROC STEPWISE) against the geographic and climatic independent variables for seed origins to identify the best model for explaining patterns of geographic variation. Cluster analysis (Ward's method in PROC CLUSTER) helped to delineate different black cherry regions of closely related provenances, and prediction ratios (PR) provided measures of association among clusters. The prediction ratios were computed from coefficients of determination (R^2) using the formula

$$PR = (1 - R^2).$$

RESULTS AND DISCUSSION

Survival

Fifth-year survival averaged 86 percent for the study, and it ranged from 63 to 100 percent among the provenances (Table 1). Variation among

Table 1. Means and ranges of tree traits in black cherry provenance test.

Statistic	Survival (%)	DBH (inches)	Height (feet)	Black Knot (% infected)
Study Mean	86	0.29	5.8	30
Range in Prov. Means	63-100	0.02-1.42	2.9-12.6	7-91

provenances was significant, but the variation among families within provenances was not significant.

Four provenances in the northeast region (#8, #10, #19, and #43) and two in the extreme south (#76 and #80) suffered more mortality than those from the central region. The only exception in the central region was #28 from Texas County in Missouri, which had only 69 percent survival. Although the pattern of geographic variation for survival was not conspicuous in the present study, it appeared similar to the one observed in earlier provenance tests (Genys and Cech 1975, Earnst 1978, Cech and Carter 1979). All of those tests used the same seed sources, and survival was measured at older ages than reported here.

Diameter and Height Growth

Average dbh and tree height were 0.29 inches and 5.8 feet, respectively, after five growing seasons at the Mississippi site (Table 1). Provenance #77 (Columbia, FL) was the fastest growing provenance and was 12.6 feet tall with a dbh of 1.44 inches. Trees from McKeen County in Pennsylvania had the poorest growth, with a fifth-year height of 2.9 feet. Provenance variation was significant for both dbh and height, but family variation within provenances was not. The lack of family variation within

provenances may be due to the very small number of families (one or two) that represented nearly half of the provenances.

Diameter and height growth decreased with increasing latitude of the provenance (Figures 2 and 3). Provenances from near the seacoast grew

Figure 2. Geographic variation for tree height at age 5 in black cherry.

Figure 3. Geographic variation for dbh at age 5 in black cherry.

faster than interior provenances at the same latitude. Within the interior, a pattern of poor growth was observed for provenances from near the Mississippi River and from the Blue Ridge and Appalachian Mountains.

One might have anticipated that provenances from milder climates than the central Mississippi planting site (i.e., from south of the site and/or closer to the ocean than the site) would grow faster than provenances from north of the site or from higher elevations, since this has been observed for other hardwood species (Webb 1970). The poor performance of the provenances in the lower Mississippi River valley is unexpected, however, and is opposite to trends observed by Land (1981) for sycamore (Platanus occidentalis L.) and by Mukewar and Land (1987) for typical southern red oak (Quercus falcata Michx. var. falcata). The four poor-performing Mississippi provenances-#62 from Marshall County, #63 from Carroll County, #82 from Amite County, and #84 from Scott County--all come from areas with thin loess soils that are similar to the soils at the planting site. Migration of alleles from farther north by seed movement down the river seems unlikely, since the species does not grow in the alluvial bottomlands of the southern Mississippi River valley. Perhaps an edaphic ecotype occurs on this margin of the species range, as suggested for sweetgum (Liquidambar styraciflua L.) by Wells et al. (1979).

Black Knot Disease Infection

Incidence of black knot disease was as high as 91 percent in the seed source from Texas County in Missouri with an overall average of 30 percent (Table 1). The lowest infection of seven percent was recorded in the Mississippi provenances from Carroll and Scott Counties. Provenances from Florida and from most of the northern origins suffered heavy infection, while five out of the six seed sources from Mississippi were least affected. Provenances from the Atlantic and Gulf coastal plains (except those from Mississippi) showed a uniform reaction to the disease, with an overall average of 25 percent infection. These differences among provences for disease susceptibility were highly significant.

Two possible explanations for the pattern of variation in black knot susceptibility are: (1) reduced vigor due to off-site planting may act as a predisposing factor for disease infection, or (2) a different physiological race of the disease organism may be present in the region of the test site, so that only the local provenances have developed resistance to that race. The pattern also supports the proposal of a local edaphic ecotype of black cherry, as suggested for the growth results.

Geographic Genetic Variation

The canonical correlation procedure indicated that the growth traits are highly correlated with all the climatic variables measuring temperature and precipitation, except the sum of growing degree days (SGROWDD) and precipitation for the 100 days after last 32°F in the spring (PREC100) (Table 2). Growth-trait correlations with latitude are large and negative,

Table 2. Correlations between dependent and predictor variables in black cherry provenance test.

Predictor Variables	Dependent Variables			
	Survival	dbh	Height	Black Knot
MANTEMP	0.03	0.75	0.81	-0.22
MAXTEMP	0.06	0.74	0.80	-0.18
MINTEMP	0.00	0.74	0.80	-0.25
MANPREC	0.04	0.51	0.58	-0.11
SMOPREC	-0.36	0.75	0.76	-0.03
ANHTDD	-0.04	-0.74	-0.80	0.20
ANCLDD	0.03	0.74	0.80	-0.27
MFRFRD	-0.03	0.74	0.80	-0.27
MDTOL32	-0.01	-0.71	-0.77	0.27
PREC100	0.33	0.13	0.21	-0.12
SGROWDD	0.21	0.18	0.18	0.10
Latitude	-0.06	-0.75	-0.81	0.18
Longitude	0.45	-0.12	-0.04	-0.09
Elevation	-0.07	-0.64	-0.69	0.31

while the correlations with elevation are moderate and negative. There is no relationship, however, between the growth traits and longitude. Survival and black knot disease have very poor or no relationship with any of the fourteen predictor variables. Tree height and mean annual temperature are the most important dependent and independent variables, since they ranked highest for the standardized canonical coefficients in the first canonical correlation. This first canonical correlation was 0.97 and accounted for 94 percent of the combined variation.

The standardized first principal component, a weighted combination of the four dependent variables, expresses 99 percent of the combined variables' variation among provenances. The pattern of variation of this derived variable is similar to the ones observed for the growth traits. When it is used as a single dependent variable in stepwise regression, latitude and mean annual temperatures of provenance are equally good as single predictors in explaining geographic genetic variation. However, the best model containing two predictor variables (annual cooling degree days and the sum of monthly precipitation during the summer) is much better than either of these single-predictor models and the best three-variable model (latitude, the sum of monthly precipitation during the summer, and elevation) is slightly better than the two-variable model. The predictive capabilities of these two larger models are 83 and 85 percent, respectively.

Since mean annual temperature or annual cooling degree days are closely related to latitude and elevation (correlations vary between -0.76 and-0.97), temperature at the seed origin may be the primary natural selection force influencing geographic genetic variation in the species. Precipitation during summer months is the next most important predictor in the models, indicating that degree of water stress during the growing season is also an influential factor. The provenances with slower growth variation from the lower Mississippi River valley and from the Appalachian highlands are, in fact, from areas of low precipitation during summer months (Figure 4b). The Appalachian highlands are also cooler than the adjacent provenances on the same latitude (Figure 4a). Therefore, the geographic patterns observed for growth traits may be explained as adaptations to temperature and summer precipitation, rather than by edaphic ecotypes or migration of alleles down the river systems.

Cluster Analysis

Cluster analysis based on fifth-year survival, growth, and black knot susceptibility when planted in central Mississippi identified two main groupings of provenances in the black cherry range: a southern group for the southeastern and southern coastal plains (approximately) and a northern group for the piedmont, mountains, and interior highlands (Figures 5 and 6). The only deviation of these groupings from the stated physiographic divisions is in the Mississippi River valley, where the northern group cuts across a northern extension of the coastal plain.

Further subdivision of each main group indicated three clusters of similar provenances in the south and four clusters in the north (Figure 5 and 6). The three southern clusters are geographically mutually exclusive

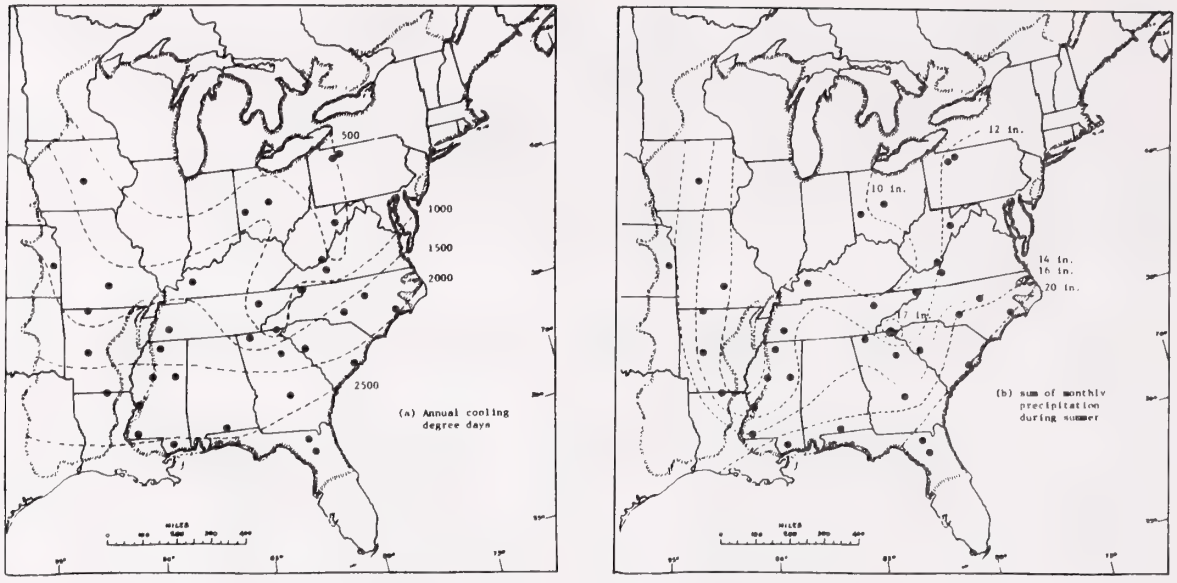


Figure 4. Geographic patterns of two climatic measures of temperature and moisture that influence provenance variation in black cherry.

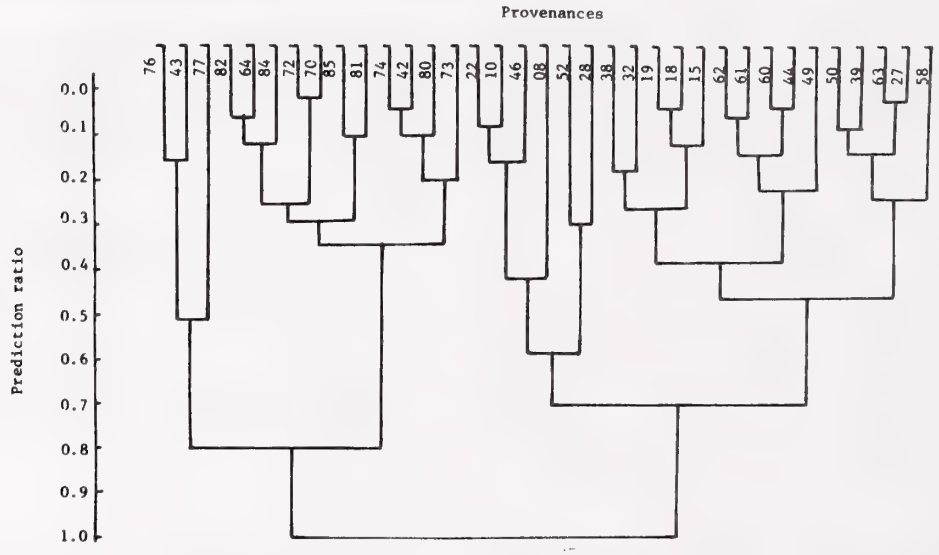


Figure 5. Output of cluster-analysis in the form of a dendrogram for black cherry provenances.

and are roughly defined as (1) northern Florida, (2) the southeastern Atlantic and lower Gulf coastal plains, and (3) the central Gulf coastal plain. Two of the northern clusters are mutually exclusive of each other and approximate (1) a central interior and (2) a northern interior region. The other two northern clusters are geographically dispersed throughout the central interior and northern interior regions. One of these dispersed groups is represented by five high elevation provenances (#8, #10, #22, #46, and #52 in Figure 6) in the eastern part of the Appalachian Highlands and one high elevation provenance (#28) in the Ozark Plateau. The other

Figure 6. Clusters of closely related provenances of black cherry.

dispersed group contains two high elevation provenances from the Valley and Ridge physiographic area of the Appalachian Mountains (#39 and #50), one high elevation provenance from eastern Kansas (#27), and two low elevation provenances from the northern extension of the coastal plain along the Mississippi River (#58 and #63).

Each of the seven clusters represents a unique combination of survival, growth, and black knot disease susceptibility (Table 3). Among the three clusters of the southern region, survival increases while growth rate and disease susceptibility decrease from cluster #1 through cluster #3. Differences in the amount of precipitation during the summer are more conspicuous than the differences in annual cooling degree days and elevation. Survival and disease susceptibility are usually higher for the northern clusters than for the southern ones, and average growth of these northern clusters closely follow the differences in annual cooling degree days. Exceptions are found in northern clusters #6 and #7, which are geographically dispersed. Cluster #6 has unusually low survival when planted in central Mississippi, while cluster #7 exhibits a very low incidence of black knot.

The dispersed cluster #7 provides an example of why cluster analysis should be used only with other supporting multivariate analyses in biosystematic studies of a species. Since it clusters provenances by similarities in dependent traits, it tells nothing about the factors influencing those similarities. Cluster #7 has slow-growing provenances with low disease incidence, so that it is different from the fast-growing southern clusters and from the disease-susceptible northern clusters. However, the factors contributing to the slow growth are different for the Mississippi River valley sources than for the Appalachian sources, as discussed earlier. Thus, the five sources within cluster #7 are probably genetically diverse. These biosystematic studies require careful examination of data by several

Table 3. Cluster means for black cherry provenances.

Cluster Names	Dependent Variables: Survival (%)	dbh (inches)	Height (feet)	Black Knot (% infected)	Independent Variables: Annual Cooling Degree Days	Summer Precipitation (inches)	Elevation (feet)
SOUTHERN REGION							
1. Northern Florida	70	0.96	10.1	44	2484	20.8	107
2. Southeastern Atlantic & Lower Gulf Coastal Plains	81	0.56	7.9	24	2073	16.5	353
3. Central Gulf Coastal Plain	90	0.42	6.9	16	2172	13.1	414
NORTHERN REGION							
4. Central Interior	96	0.23	5.6	37	1531	13.5	1142
5. Northern Interior	93	0.04	3.9	45	898	11.3	1078
6. Appalachian & Ozark Highlands	75	0.08	4.0	53	859	12.1	1910
7. High & Low Elevation Group	94	0.18	4.9	15	1431	12.2	1216

analyses procedures, including canonical correlations, principal components, stepwise regression, and cluster analyses, to help identify taxonomic groups and causal factors. The proposed groups must then be verified by biochemical and cytogenetic investigations.

CONCLUSIONS

Provenance variance is significant for survival, tree height, dbh, and black knot disease susceptibility in black cherry when planted in central Mississippi. Seed sources from near the Gulf and Atlantic sea coasts grow faster than those from interior regions. Seed sources from Mississippi and from some of the high elevations in the central and northern regions have above-average resistance to black knot disease. Annual cooling degree days and the sum of monthly precipitation during the summer are the best two variables for explaining the observed geographic genetic variation in the species. Seven groupings (or clusters) of provenances in the study reported here can be identified, based on similarities in survival, growth, and disease resistance. The five of these which are geographically mutually exclusive may be tentatively defined as "provenances", but must be verified by biochemical and cytogenetic investigations. Abnormally slow growth from sources in the lower Mississippi River valley is apparently an adaptation to high water deficits during the summer rather than an edaphic ecotype or a reflection of migration of alleles down the river system.

LITERATURE CITED

Barnett, P. E. 1977. Black cherry seed source study in northern Alabama--ten year results. Proc. 14th So. For. Tree Improv. Conf., p. 201-205.

Cech, F. C., and K. K. Carter. 1979. Geographic variation in black cherry: Ten-year results of a West Virginia provenance test. Proc. 1st North Central Tree Improv. Conf., p. 21-27.

Cech, F. C., and J. H. Kitzmiller. 1968. Geographic variation in seed and seedling characteristics of black cherry. Proc. 15th N.E. For. Tree Improv. Conf., p. 53-60.

Earnst, R. L. 1978. Progress report: Black cherry provenance trial field planting. USDA Forest Service, Northeastern Forest Experiment Station, Warren, PA. 12 pp.

Genys, J. B., and F. C. Cech. 1975. Intraspecific variation among 25 populations of black cherry (P. seretina) studied near Washington, D.C. Proc. 22nd N.E. For. Tree Improv. Conf., p. 176-184.

Land, S. B., Jr. 1981. Genetic variation, heritabilities, and selection strategies for early growth of sycamore in the Gulf South. Proc. 16th So. For. Tree Improv. Conf., VPI&SU, Blacksburg, VA. p. 123-135.

Mukewar, A. M., and S. B. Land, Jr. 1987. Geographic genetic variation in southern red oak. Proc. 19th So. For. Tree Improv. Conf. (In Press.)

Pitcher, J. A. 1984. Geographic variation patterns in seed and nursery characteristics of black cherry. USDA Forest Service, Southern Forest Experiment Station, New Orleans, LA. 8 pp.

SAS Institute. 1985. SAS user's guide: statistics, Version 5 ed. SAS Institute, Inc., Cary, N.C. 956 pp.

Webb, C. D. 1970. Genetics and tree improvement in the oak-yellow poplar type. In: The Silviculture of Oaks and Associated Species. USDA For. Service Res. Paper NE-144. p. 57-66.

Wells, O. O., G. L. Switzer, and W. L. Nance. 1979. Genetic variation in Mississippi sweetgum. Proc. 15th So. For. Tree Improv. Conf., Mississippi State University, Mississippi State, MS. p. 22-32.

Wright, J. W., and W. A. Lemmien. 1974. Seven-year results from black cherry and black walnut provenance tests in Michigan. Proc. 8th Central States For. Tree Improv. Conf., p. 61-64.

MODELING DISTRIBUTIONS OF STEM CHARACTERISTICS OF GENETICALLY IMPROVED LOBLOLLY PINE

J. E. Janssen [1/] and P. T. Sprinz [2/]

Abstract.--Diameter distribution models for genetically improved and unimproved loblolly pine were developed using the moment-based beta probability density function. The parameters of the models were based on stand age, stand density, and average height of the dominants. Comparisons were made between the improved and unimproved predicted distributions to determine the effect of genetic improvement on stand growth and yield. Preliminary results indicate that diameter distributions of improved females are less peaked and are shifted toward larger diameter classes than the unimproved females.

Additional keywords: Pinus taeda, tree improvement, diameter distributions, growth and yield.

The advancement of tree improvement, that is, increasing productivity with the use of genetically improved stock, has made considerable gains in improving individual tree characteristics. However, knowledge regarding the effect of genetic variation on stand growth and yield at harvest age is limited. Yield tables that are currently available apply only to natural stands or plantations established from "woodsrun" stock. It is essential that existing growth and yield models be modified to incorporate the effects of genetic improvement if reliable estimates of genetic improvement at a stand level are to be made.

In recent years, experiments established in the past have reached sufficient age for the long term effects of genetic improvement to be tested. For this study, individual tree data collected from half-sib family-block plantings of rotation-aged stands of genetically improved loblolly pine (Pinus taeda L.) established by the Texas Forest Service were used to model diameter distributions over time. These distributions were then compared to diameter distributions of unimproved loblolly pine grown in the same plantings. Quantification of the similarities and differences in the shapes and levels of the distributions through time for the genetically improved and unimproved loblolly pine provided additional understanding of the development of stands of improved stock and information for modeling their growth.

[1/] Graduate Research Assistant, Department of Forest Science, Texas A&M University, College Station, TX 77843.

[2/] Assistant Professor, Department of Forest Science, Texas Agricultural Experiment Station, Texas A&M University System, College Station, TX 77843.

DATA

Three loblolly pine plantations (001, 006, 041), in which the trees were planted in a replicated randomized block design, were included in the research. Plantations 001 and 041 were established in 1952-53 and 1955-56, respectively, in Many, LA at the A. J. Hodges Experiment Area. The measurement plots were composed of the inner 64 trees of 100 trees per plot planted in a 7 by 7 foot spacing. In plantation 001, there were twelve females, comprising six improved females and six unimproved females, and in plantation 041 there were four females, two improved and two unimproved females. Plantation 006 was established in 1956-57 in Fastrill, TX at the Arthur H. Temple Research Area, with thirty females, fifteen improved and fifteen unimproved. In each of the plantations, the mother trees for the seed sources were found in the same proximity; the improved and unimproved females were then planted side by side for paired comparisons.

The measurements taken consisted of individual tree diameter outside bark at breast height and total height at ages 1, 2, 3, 5, 10, 15, 20, and 30 from planting. Plantation 001 was thinned at ages 10 and 17, plantation 006 at age 13, and plantation 041 at age 14.

METHOD OF ANALYSIS

The frequency distributions of diameter measurements have been described using probability density functions (pdf). An essential feature in using pdf's is the ability to predict the pdf parameter values for a given set of stand conditions. In the past many different probability distributions have been used. For this study, the beta distribution was chosen since it can assume a variety of shapes, can be easily fit by moments, and has been utilized successfully in several previous growth and yield studies. Yield tables based on the beta distribution have been prepared by Bennett and Clutter (1968), Beck and Della-Bianca (1970), Lenhart and Clutter (1971), and Lenhart (1972).

The beta distribution, as presented by Krutchkoff (1970), is of the form:

$$f(x) = \begin{cases} \dfrac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\ \Gamma(\beta)}\ x^{\alpha-1}\ (1-x)^{\beta-1} & 0<x<1 \text{ and } \alpha,\beta>0 \\ 0 \text{ otherwise} \end{cases} \qquad (1)$$

Since the family of beta distributions is a family of probability densities of continuous random variables taking on values in the interval (0,1), the previous formula must be modified to account for diameter data in the interval (Dmin, Dmax). The following equation is used to code the tree diameters (Di) so that all diameters in a plot fall in the range of the beta distribution (0<Xi<1):

$$Xi = \frac{Di - Dmin}{Dmax - Dmin} \qquad (2)$$

where,

Xi = scaled tree diameter
Di = unscaled tree diameter
Dmin = minimum tree diameter observed on a particular plot
Dmax = maximum tree diameter observed on a particular plot

Thus, the following modification of equation (1) results:

$$f(D) = \begin{cases} \left[\frac{\Gamma(\alpha+\beta)}{(Dmax - Dmin)\Gamma(\alpha)\Gamma(\beta)}\right] \left[\frac{D - Dmin}{Dmax - Dmin}\right]^{\alpha-1} \left[\frac{D - Dmin}{Dmax - Dmin}\right]^{\beta-1} \\ 0 \text{ otherwise} \end{cases} \quad (3)$$

$Dmin<D<Dmax;\ \alpha,\beta>0$

where,

f(D) = the relative frequency of occurrence of a given diameter, D
α,β = model parameters to be estimated from the data
Dmin, Dmax as above

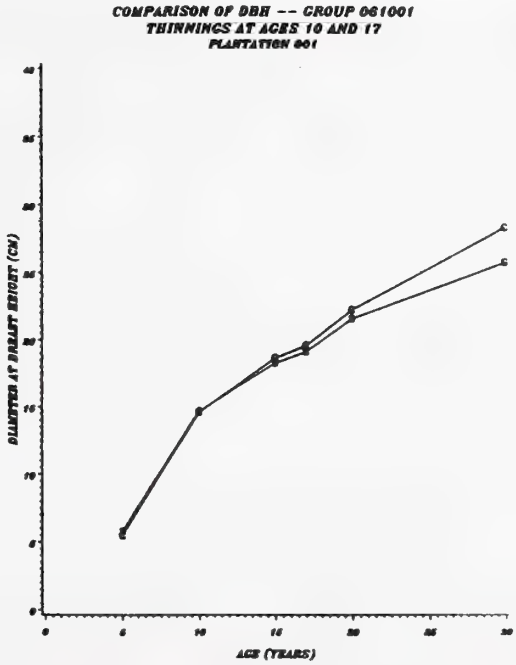
To determine the parameters of the beta distribution, α and β, the method of moments was used instead of the method of maximum likelihood. This was because moment estimates are easier to compute and both Lloyd (1966) and Strub (1972) found that relatively small differences existed between the parameter estimates of the two techniques. The method of moment equations were computed from data grouped into 1-centimeter class intervals. The first and second noncentral moments of x (or diameter) were estimated by the average diameter of the stand and the basal area per acre. Once the parameter estimates for the beta distribution were made on the 56 plots, the predicted diameter distribution was compared to the observed diameter distribution by means of the X^2 Goodness-of-Fit test.

The moment estimates of the parameters α and β were predicted for each plot using stand age, average height of the dominant trees, number of trees per hectare, and genetic information. Average height of the dominant trees was used in place of site index since height was a measured variable. Average height of the dominant trees for the unimproved females was also tested. The genetic information was included by means of coding each of the 20 different females for the three plantations combined as genetically improved or unimproved. This was done by means of indicator variables. Each group of a genetically improved (superior) female and unimproved (check) female was tested independently of the other groups. Numerous models were tested using different combinations of the stand conditions, including Burkhart and Strub's (1974) parameter prediction equations for estimating α and β of the beta pdf. Dmin and Dmax were also predicted by multiple regression techniques using the same independent variables of stand age, average height of the dominant trees, and number of trees per hectare.

The regression models were analyzed using fit and prediction statistics. Fit statistics, such as the F-values, the coefficient of determination (R^2), the sum of square error (SSE), and the mean squared error (MSE), were used to determine how well the data conformed to the model. Prediction statistics, such as PRESS, Cp, and VIF, give an indication of a model's predictive ability. The PRESS (Predictive Error Sum of Squares) evaluates alternative models when the objective is prediction. The model which yields the lowest PRESS value may predict better than the other models. The Cp criterion evaluates the bias of the regression model, and the variance inflation factor (VIF) indicates if there is multicollinearity. Residuals, the deviation between data and fit, were plotted to detect any other model inadequacies.

RESULTS AND DISCUSSION

The following results are given for one grouping of a superior family and a related check family. Similar results were found for the other groups. Comparisons of the average diameter at breast height and the average height of the dominants at each age for the superior and check families are shown (Figures 1, 2a). Comparisons of the number of trees per hectare, the basal area per hectare, and volume per hectare for the same group are also shown (Figure 2b, c, and d).



S = SUPERIOR C = CHECK

Figure 1. Comparison of the superior versus check female for average diameter at breast height.

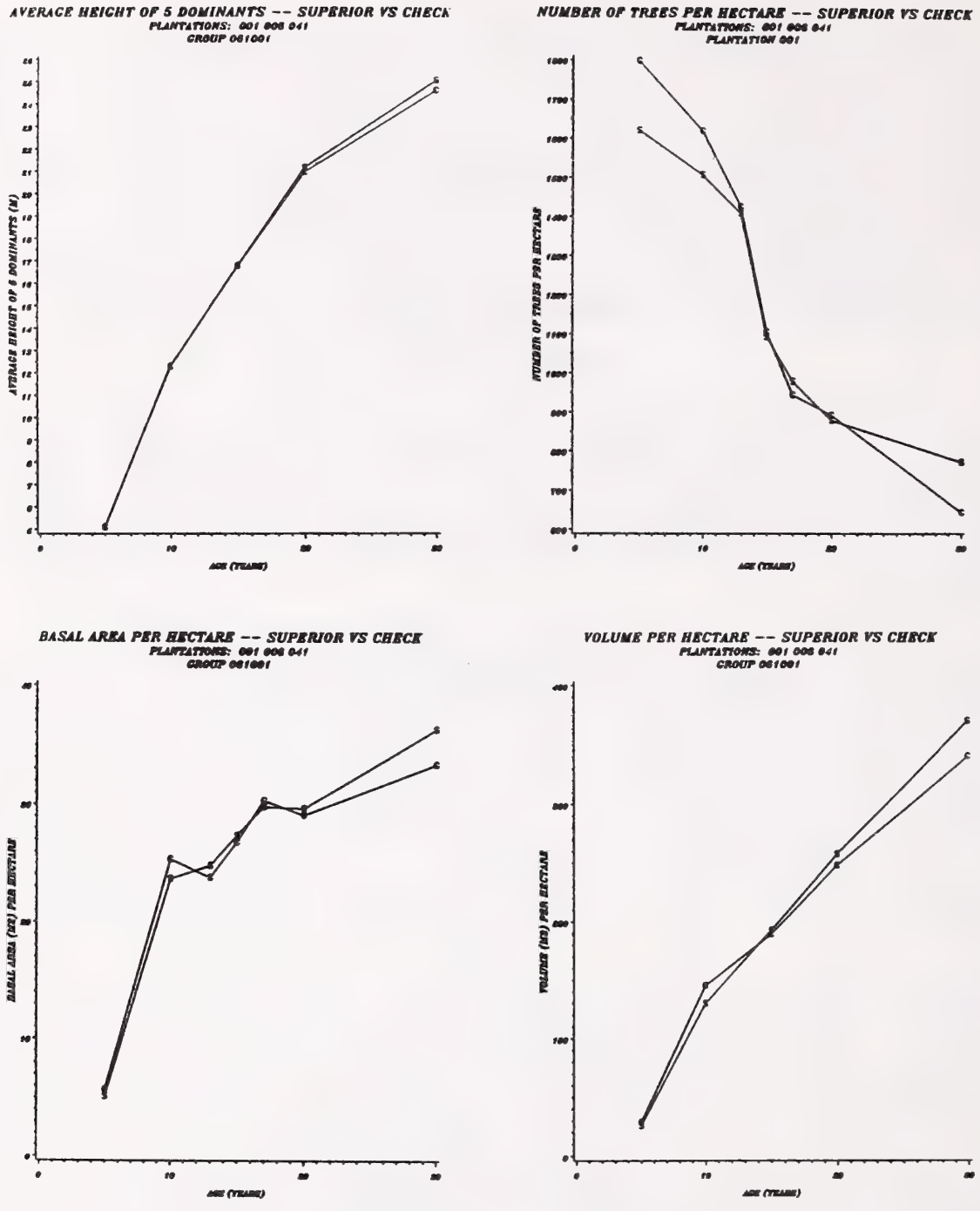

AVERAGE HEIGHT OF 5 DOMINANTS -- SUPERIOR VS CHECK
PLANTATIONS: 001 006 041
GROUP 061001
AVERAGE HEIGHT OF 5 DOMINANTS (M)
AGE (YEARS)
NUMBER OF TREES PER HECTARE -- SUPERIOR VS CHECK
PLANTATIONS: 001 006 041
PLANTATION 001
NUMBER OF TREES PER HECTARE
AGE (YEARS)
BASAL AREA PER HECTARE -- SUPERIOR VS CHECK
PLANTATIONS: 001 006 041
GROUP 061001
BASAL AREA (M2) PER HECTARE
AGE (YEARS)
VOLUME PER HECTARE -- SUPERIOR VS CHECK
PLANTATIONS: 001 006 041
GROUP 061001
VOLUME (M3) PER HECTARE
AGE (YEARS)
S = SUPERIOR
C = CHECK

Figure 2. Comparison of the superior versus check female for a) average height of the dominants, b) number of trees per hectare, c) basal area per hectare, and d) volume per hectare.

Observed values of Dmin, Dmax, average diameter, and average squared diameter were used to solve for the parameters, α and β, of the moment-based beta distribution. A solution was achieved for each of the 56 plots. Using the stand conditions, age, height of the dominants of the unimproved females (Chtd), and trees per hectare (Tph) from each sample plot, stand average attributes, Dmin, Dmax, average diameter, and average squared diameter, were predicted by linear regression equations and parameters recovered.

The models to predict the parameter estimates were chosen on the basis of the fit and prediction statistics as described in the method of analysis. These models were the same as the models determined by Burkhart and Strub (1974). They are:

$$\hat{\alpha} = \alpha_0 + \alpha_1 (\text{Age/Tph}) + \alpha_2 (\text{Age})(\text{Chtd})$$

$$\hat{\beta} = \alpha_0 + \alpha_1 (\text{Age/Tph}) + \alpha_2 (\text{Tph})(\text{Chtd})$$

$$\hat{\text{Dmin}} = \alpha_0 + \alpha_1 (\text{Chtd}) + \alpha_2 (\text{Age})(\text{Tph}) + \alpha_3 (\text{Chtd/Tph})$$

$$\hat{\text{Dmax}} = \alpha_0 + \alpha_1 (\text{Chtd}) + \alpha_2 (\text{Age})(\text{Tph}) + \alpha_3 (\text{Chtd/Tph})$$

The full versus reduced F-tests for differences in intercept and/or slope between the superior and check females indicated that there were no significant differences in either intercept or slope for the parameters α and β. No significant differences between intercept and slope were indicated for Dmin, but significant differences at the .05 alpha level were detected for Dmax in both intercept and slope.

The superior and check females were then combined and estimates of α and β were obtained for each plot from the parameter prediction equations. Using the estimates of α and β, the predicted diameter distributions produced by the moment-based beta using actual average stand attributes were determined for each plot. The observed and predicted diameter distributions using stand attributes were similar (Figure 3).

A comparison of the predicted diameter distributions using stand statistics for the superior and related check females provided information on the development of stands of improved stock. There were two main cases resulting: 1) that the distributions of the superior females had lower peaks and were shifted to the right, as seen in Figure 4a, and 2) that the distributions of superior females had higher peaks and were shifted slightly to the right, as seen in Figure 4b. Both graphs indicate that there is definitely a difference in the average maximum diameter, as indicated by the F-tests. This is an important result, since it indicates that stands of genetically improved females may have distributions with trees of greater diameters.

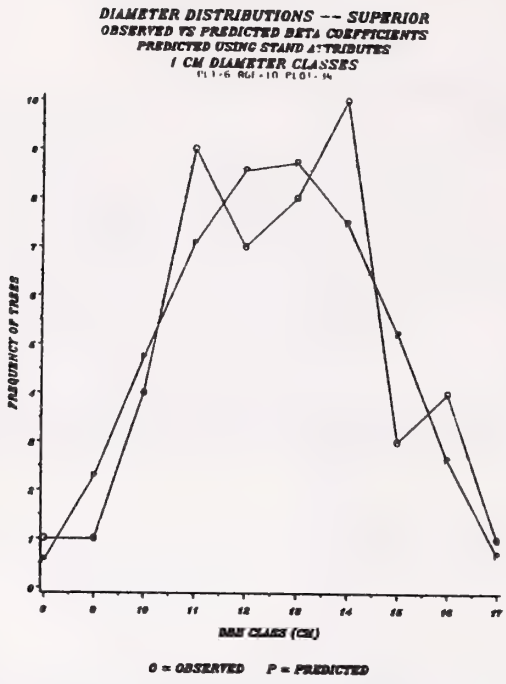

DIAMETER DISTRIBUTIONS -- SUPERIOR
OBSERVED VS PREDICTED BETA COEFFICIENTS
PREDICTED USING STAND ATTRIBUTES
1 CM DIAMETER CLASSES
FREQUENCY OF TREES
DBH CLASS (CM)
O = OBSERVED P = PREDICTED

Figure 3. The observed versus the predicted diameter distribution using stand average attributes for a particular plot.

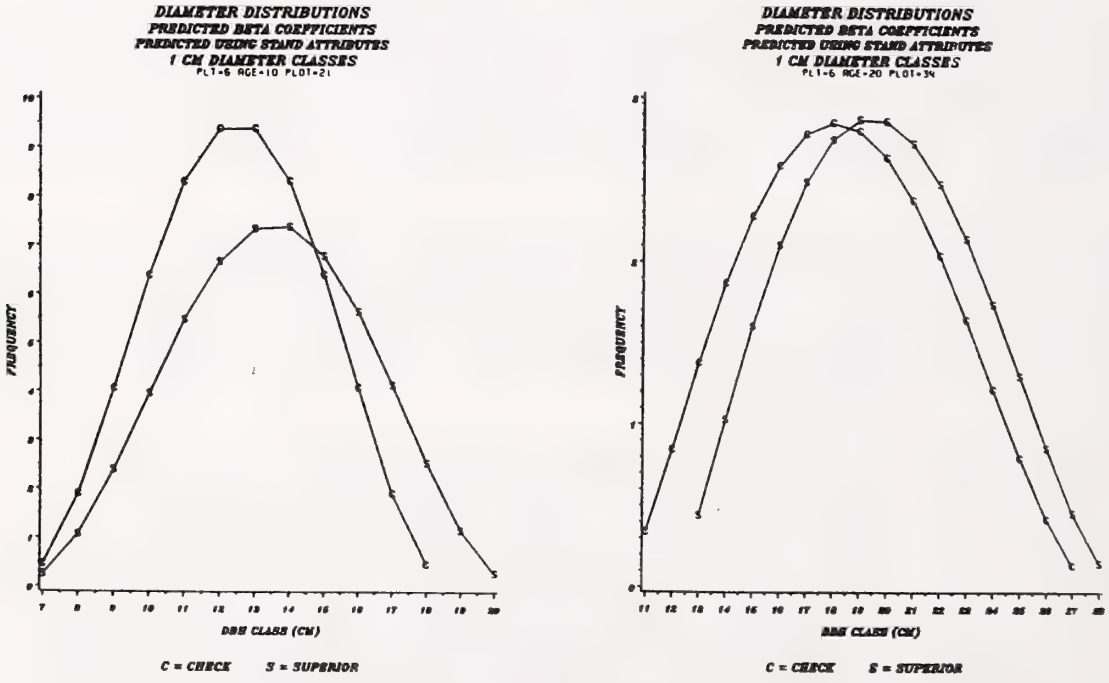


Figure 4. Comparisons of the predicted diameter distribution using stand attributes for a superior versus check female, where a) shows the first relationship and b) shows the second relationship.

CONCLUSIONS

The effect of genetic improvement on the development of diameter distributions of loblolly pine at harvest age has been tested for a limited sample of genetically improved and unimproved females. The comparisons in Figure 4 indicated that there are differences in the peaks and shapes of the distributions. The average maximum diameter for the improved females was found to be larger. These results are preliminary. Further models and comparisons will be tested. In addition, two different probability density functions will be used, the Weibull and Johnson's Sb. The Weibull function has been utilized in several recent yield studies and has also been successful in quantifying diameter distributions. Johnson's Sb has not been used as extensively in growth and yield studies, but was found to have desireable characteristics in comparison to other distributions (Hafley and Schreuder 1977).

Comparing the results of the three different distributions will more accurately indicate the differences between the genetically improved and unimproved loblolly pine diameter distributions. Additional quantification of the effects of genetic improvement of the diameter distributions through time will provide increased understanding of the development of stands of improved stock and information for modeling their growth.

LITERATURE CITED

Beck, D. E., and L. Della-Bianca. 1970. Yield of unthinned yellow-poplar. U. S. For. Serv. Res. Paper SE-58, 20 p.

Bennett, F. A., and J. L. Clutter. 1968. Multiple-product yield estimates for unthinned slash pine plantations -- pulpwood, sawtimber, gum. U. S. For. Serv. Res. Paper SE-35, 21 p.

Burkhart, H. E., and M. R. Strub. 1974. A model for simulation of planted loblolly pine stands. IN: Growth Models for Tree and Stand Simulations (Ed. J. Fries). Royal College of Forestry, Stockholm, Sweden. p. 128-135.

Hafley, W. L., and H. T. Schreuder. 1977. Statistical distributions for fitting diameter and height data in even-aged stands. Can. J. For. Res. 7:481-487.

Krutchkoff, R. G. 1970. Probability and statistical inference. Gordon and Breach, New York, N. Y. 291 p.

Lenhart, J. D. 1972. Cubic-foot yields for unthinned old-field loblolly pine plantations in the Interior West Gulf Coastal Plain. Stephen F. Austin State Univ., School of Forestry, Texas Forestry Paper No. 14, Nacogdoches, TX. 46 p.

Lenhart, J. D., and J. L. Clutter. 1971. Cubic-foot yield tables for old-field loblolly pine plantations in the Georgia Piedmont. Ga. For. Res. Coun. Report 22 -- Series 3, Macon, Ga. 12 p.

Lloyd, F. T. 1966. A description of Virginia pine diameter distributions. M. S. thesis, Virginia Poly. Inst. and State Univ., Blacksburg, Va. 46 p.

Strub, M. R. 1972. A beta distribution approach to diameter class frequencies in planted loblolly pine stands. M. S. thesis, Virginia Poly. Inst. and State Univ., Blacksburg, Va. 68 p.

JUVENILE-MATURE CORRELATIONS IN SYCAMORE

E. B. Schultz[1]/ and S. B. Land, Jr.[2]/

Abstract.--Genetic and simple correlations for five seed traits, two nursery traits, and four early field traits were made with fifth year DBH, height, dry weight, and volume in a sycamore progeny test. Germination value and first year root-collar diameter were identified as potential early selection traits for increasing gain per unit time in fifth-year volume. Families that showed good rank stability over time were also observed as stable over progeny test locations. Selection for both stability and performance may be the most desirable strategy for advanced-generation breeding programs.

Additional keywords: Platanus occidentalis, early selection, stability.

Correlations between juvenile and mature traits are receiving increased consideration from tree breeders because of the economics of shortening breeding cycles and increasing gain per unit time. Since genetic correlations between early and mature traits are rarely perfect, correlated gain from indirect selection on early traits for improvement of mature traits is usually less than gain from direct selection on mature traits for a given breeding cycle. However, increased gain per unit time has been demonstrated where several breeding cycles can be carried out in the time required for one cycle of direct selection (McKinley and Lowe, 1986). Savings in cultural and mensurational costs can also be realized from selection of juvenile traits as opposed to mature traits.

Mature traits may be volume or dry weight at three to ten years of age in short-rotation energy crops. Juvenile traits would therefore be seed, nursery, and early field observations. The time saved in early selection for a short-rotation breeding program is small compared to a traditional breeding program but may result in considerable savings in expense of field testing the progeny (Land et al., 1987).

Genetic and simple correlations for early selection of sycamore (Platanus occidentalis) biomass production are presented. Seed, nursery seedling, and outplanted seedling traits are correlated with three- and five-year DBH, height, dry weight, and volume. Stability of family ranks over time, traits, and locations and losses due to early selection are examined. A potential breeding strategy is suggested.

1/ Research Associate, School of Forest Resources, Mississippi State University, Mississippi State, MS.

2/ Professor, Department of Forestry, Mississippi State University, Mississippi State, MS.

Contribution No. 6695 of the Mississippi Agricultural and Forestry Experiment Station.

MATERIALS AND METHODS

A large open-pollinated sycamore progeny test and a separate seed-trait study with 79 families in common were used to derive correlations between early selection and biomass traits. The 79 families represent 16 source selections made from four latitudinal transects across eastern Arkansas and Louisiana, Mississippi, and western Alabama (Figure 1). They number 18, 20, 20, and 21 families respectively from the northern to the southern transect.

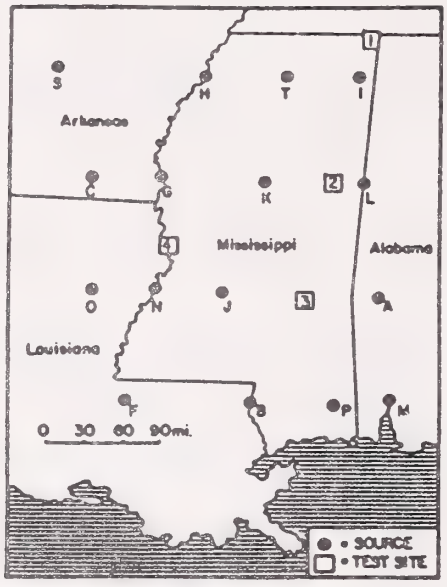


Figure 1. Locations of seed sources and planting sites used in the sycamore progeny test.

Open-Pollinated Progeny Test

The progeny test was established at four locations in Mississippi (Figure 1). A description of these sites and the early genetic estimates from the study are given by Land (1981). Two planting years, six randomized block replications per year, and three individual trees per family plot are represented at each of the progeny test locations. There is an average of 140 trees per family composing each correlation between family performance for juvenile and mature traits. Thus, family and within family sample sizes are sufficiently large to minimize problems associated with sampling error (Lambeth, 1983). The study also has the advantage of correlating family performance over different planting locations.

Traits chosen from the progeny test for use in the correlation study were nursery root-collar diameter, nursery height, first-year (in the field) height, first-year root-collar diameter, third-year DBH, third-year height, fifth-year DBH, fifth-year height, fifth-year stem plus limb dry weight, and fifth-year stem volume.

Seed Trait Study

The families for the seed trait study were taken from the same mother-tree seed collections as those used for the progeny test. Germination counts were

taken on 100 seed from each of three individual trees per family for the first, second, third, fifth, seventh, ninth, fourteenth, and twenty-first days in a germination chamber. Numbers of ungerminated seed and percent full seeds were also determined. A germination value (GV), to measure in one statistic both speed and completeness of germination was calculated for each family using the following formula (Czabator, 1962):

GV = Mean daily germination (MDG) * Peak value (PV),

where MDG = total number of seed germinated, divided by the total length of the germination period (21 days), and

PV = the maximum value of the cumulative percent germination for each day of the trial divided by the corresponding number of days from the beginning of the trial.

Unpublished data on seedball diameter and weight, weight of clean seed per seedball, and weight of 100 seed per seedball were also used for two replications of five seedballs per family. These seed and seedball traits were correlated with the progeny test traits.

Correlations

Because seed trait and progeny test studies were separate in nature (i.e. seeds were not traced from the germination chamber to the field, and experimental designs of the studies differed), clean genetic correlations from one analysis of covariance table could not be obtained. Product-moment and rank correlations (Sokal and Rolf, 1969) were calculated and considered to be composed of genetic, maternal, and common environment effects. Analyses of covariance on family means were performed for combinations of nursery and field traits in the progeny test. Components of covariance were estimated by equating actual with expected mean products, and genetic correlation estimates were obtained from those components.

Performance Levels

Performance level (PL) (North Carolina State University-Industry Cooperative, 1979) measures a family's position in the distribution for a particular trait in a given environment:

$$PL = (FM - (GM - (2 * STD))) / ((GM + (2 * STD)) - (GM - (2 * STD)))$$

where FM = family mean
GM = grand mean
STD = standard deviation.

Performance levels from different locations are averaged to obtain a single PL value for each family and trait. These values range from 0 to 100, with 50 representing a performance level of an average family.

PL values were used in the present study to make adjustments in family performance over varying environments in the progeny test. The Vicksburg progeny test site (location 4, Figure 1) produced site means that were sometimes twice as large as any other site, so that family averages across sites would be biased by performance at this site.

Product-moment correlations of all traits were made on the performance level of each family for that trait as opposed to the measured trait itself.

RESULTS AND DISCUSSION

Correlations

Product-moment correlations between seed traits and third and fifth-year progeny test traits were only significant for germination value and percent full seeds (Table 1). Germination value was significantly correlated with DBH, volume, and dry weight; however it was not correlated with height. Percent full seeds gave significant correlations with third- and fifth-year DBH only. Consistent correlations of 0.3 for germination value were considered to be quite good, since other early selection studies (Robinson et al., 1984; Lambeth et al., 1982) have indicated insignificant seed trait correlations with field traits. Germination value may be a better measure of seed vigor than the traditionally used germination percent or rate, and therefore, correlated well with seedling and mature plant vigor.

Table 1.--Product-moment correlations between the seed study and progeny test for performance level traits for 79 sycamore families.

Biomass Rotation Trait	Germination Value	% Full Seeds	Seedball			
			Diameter	Dry Wt.	Wt. of Clean Seed	Wt. of 100 Seed
3rd-Year DBH	0.327**	0.243*	-0.086	0.037	0.050	0.073
3rd-Year Height	0.157	0.063	-0.081	-0.007	0.005	0.038
5th-Year DBH	0.338**	0.221*	-0.158	-0.132	-0.156	-0.172
5th-Year Height	0.159	0.039	-0.225*	-0.173	-0.166	0.062
5th-Year Stem/ Limb Dry Wt.	0.305**	0.166	-0.177	-0.149	-0.169	-0.161
5th-Year Stem Volume	0.310**	0.173	-0.175	-0.147	-0.167	-0.161

* Significant at the 0.5 level
** Significant at the 0.1 level or above

All seedball traits (Table 1) and nursery root-collar diameter (Table 2) were usually not correlated with field traits. A tendency for the estimates to be negative was noted. Negative correlations for root-collar diameters in the greenhouse with fifth-year height, diameter, and volume in the field has been reported by Robinson et al. (1984) for loblolly pine. The negative relationships are thus far unexplained.

Family mean nursery height did not correlate well with any trait measured in the field, but first-year root-collar diameter and height were strongly correlated with third- and fifth-year measurements in the field (Table 2). This pattern of weak nursery and strong early field correlations suggests that

either strong family by microenvironment interactions in the nursery bed or maternal effects are influencing family performance. A study currently underway will make reciprocal controlled pollinations among selected individuals from this progeny test to determine the role of maternal effects on early selection (Land et al., 1986).

Table 2.--Estimates of genetic correlations and product-moment correlations (in parentheses) between early and three- and five-year performance level traits from a sycamore progeny test.

Biomass Rotation Trait	Nursery Root Collar	Nursery Height	1st-Year Root Collar	1st-Year Height	3rd-Year DBH	3rd-Year Height
3rd-Year DBH	-0.335 (-0.012)	0.257 (0.219)**	0.617 (0.631)**	0.638 (0.630)**	-	-
3rd-Year Height	-0.420 (-0.079)	0.136 (0.150)	0.194 (0.395)**	0.638 (0.631)**	-	-
5th-Year DBH	-0.178 (-0.010)	0.161 (0.134)	0.641 (0.578)**	0.630 (0.517)**	0.973 (0.920)**	0.715 (0.756)**
5th-Year Height	-0.471 (-0.123)	0.096 (0.099)	0.335 (0.393)**	0.761 (0.563)**	0.830 (0.790)**	0.891 (0.888)**
5th-Year Stem/ Limb Dry Wt.	-0.072 (-0.004)	0.265 (0.164)	0.721 (0.565)**	0.829 (0.557)**	1.019 (0.903)**	0.804 (0.793)**
5th-Year Stem Volume	-0.076 (-0.013)	0.084 (0.167)	0.727 (0.564)**	0.841 (0.561)**	1.029 (0.911)**	0.803 (0.797)**

** Significant at the .01 level or above

The strong correlations between first-year, third-year and fifth-year measurements in the field indicate that four years could be cut from the short rotation biomass breeding cycle. Land (1981) showed that even for correlations above 0.9 dramatic increases in selection intensities must be made to compensate for the differences between indirect and direct gains. However, a savings of four years in a short rotation program could affect gain per unit time by (1) allowing early identification of genotypes to place in accelerated breeding facilities and by (2) identifying genotypes for vegetative propagation of planting stock for clonal forestry at an age when the material is still easy to root.

Losses From Early Selection

Losses in fifth-year volume performance level from early selection of the best twenty families range from fifteen percent for nursery traits to less than three percent for third-year traits (Table 3). Nursery root-collar diameter, which was negatively correlated to fifth-year volume, correctly identified five out of twenty high volume families. Five is therefore considered as a background count of families identified by chance against which other early selec-

tion traits can be compared. Germination value, whose correlation with fifth-year volume was 0.310, correctly identified one-half of the twenty families. The same number was identified by first-year height whose correlation was 0.561. One reason for the improved predictive power of germination value relative to its correlation is that the upper quartile of germination value families were better correlated with fifth-year volume than the lower 59 families. A high upper quartile correlation also played a role in the very impressive six out of ten correctly chosen families for first-year root-collar diameter.

Table 3.--Average performance levels and number of common top families selected for greatest fifth-year stem volume.

Trait	Fifth-year average volume performance level of top 20 families for each trait[a/]	Number of families common to top fifth-year stem volume families		
		Out of top		
		20 Families	10 Families	5 Families
Indirect Selection:				
Germination Value	52	10	3	2
Nursery Root Collar	51	5	2	0
Nursery Height	51	5	1	1
First-year Height	55	10	3	2
First-year Root Collar	55	9	6	1
Third-year DBH	59	16	5	3
Third-year Height	58	14	4	1
Direct Selection:				
Fifth-year Stem Volume	60	20	10	5

[a/] A difference of 10 in performance level for fifth-year stem volume roughly translates to a 13% difference in actual volume.

In consideration of both time and minimal losses, germination value and first-year root-collar diameter are the most promising traits for early selection.

Stability of Family Performance

Ranks across traits for the top twenty families in fifth-year volume indicate that two families, F101 and F205, from a southern Louisiana source showed very good rank stability for all traits (Table 4). Other families exhibited good stability for part of the traits. For example, family N109 was stable for all but first-year traits.

Table 4. --Ranks for the best twenty fifth-year stem volume families over selected traits.

	Rank out of 79 total families									
	Germination Value	First-year Root Collar	First-year Height	Third-year DBH	Third-year Height	Fifth-year DBH	Fifth-year Height	Fifth-year Stem/Limb Dry Wt.	Fifth-year Vol.	
F101	6	2	1	1	2	1	1	1	1	*
N109	2	21	24	3	9	2	2	2	2	
B110	20	41	25	12	7	3	8	3	3	
N204	4	16	40	17	24	6	7	4	4	
B209	27	9	15	2	14	4	15	5	5	
N110	12	10	13	15	38	11	9	8	6	
P110	80	4	29	13	15	8	11	7	7	
M104	55	1	9	28	30	10	6	6	8	
N102	51	7	30	7	33	5	23	9	9	
F201	17	32	4	4	1	12	4	11	10	
L110	45	29	43	9	13	7	19	10	11	
F108	23	64	20	10	12	9	5	12	12	
P101	25	55	49	29	4	15	3	13	13	
P206	67	12	6	11	5	14	10	14	14	
A109	5	42	37	5	3	19	21	15	15	
N208	8	35	48	19	36	17	14	17	16	
C109	46	23	19	16	11	18	29	16	17	
F205	18	27	14	8	18	16	18	20	18	*
G106	11	25	46	23	41	13	42	19	19	
P201	54	19	11	21	19	24	12	18	20	

* Good family rank stability from seed to nursery to field traits

The families that showed rank stability over ages and traits were also stable over progeny test sites (environments). Since it is impossible to directly predict the future rank stability of families, one might indirectly test for stability by ranking families for early traits over environments. The use of a multiple selection index, which would weight stability over environments and productive value, could be used to improve early selection in the next generation, if heritabilities are high. Even when stability over years is not indirectly predicted, it could be measured in the first generation and used to shorten the breeding cycles of the second and third generations.

SUMMARY AND CONCLUSIONS

1. Germination value was moderately correlated to fifth-year volume production. This seed trait could be used to decrease the cost of progeny testing by screening out the worst families before sowing in the nursery or greenhouse.

2. First-year height and root-collar diameter in the progeny test had high genetic correlations with fifth-year volume. These traits could be used in early selection of genotypes for an accelerated breeding program and for vegetative propagation of planting stock.

3. Families that were stable in rank over ages and traits were also stable over progeny test environments. Stability might be used as part of an early selection program to improve age-age correlations or to reduce genotype-by-site interactions.

4. Two unanswered questions are (a) the importance of maternal effects on early correlations and (b) the magnitude of correlations between seed or nursery traits and field performance at the individual genotype level. A study is underway to answer these questions.

LITERATURE CITED

Czabator, F. J. 1962. Germination value: an index combining speed and completeness of pine seed germination. For. Sci. 8(4):386-396.

Lambeth, C. C. 1983. Early testing - an overview with emphasis on loblolly pine. Proc. 17th So. For. Tree Improv. Conf. p.297-311.

Lambeth, C. C., R. W. Stonecypher, B. J. Zobel. 1982. Early testing of Douglas-fir in phytotron environments - the effect of selection trait and genotype - environment interaction. Proc. 7th No. Amer. For. Biol. Workshop. Lexington, KY.

Land, S. B. 1981. Genetic variation, heritabilities, and selection strategies for early growth of sycamore in the Gulf South. Proc. 16th So. For. Tree Improv. Conf. p.123-135.

Land, S. B., W. W. Elam, E. B. Schultz. 1986. Early selection criteria and clonal propagation methods for increased production of sycamore in short-rotation energy systems. Project Proposal, Subcontract No. 86X-95902C. Oak Ridge National Laboratory, Martin Marietta Energy Systems, Inc., Oak Ridge, TN.

Land, S. B., W. W. Elam, E. B. Schultz, F. T. Bonner, and J. A. Vozzo. 1987. Early selection and clonal propagation methods for increased productivity of sycamore in short-rotation energy systems. Annual Report. Oak Ridge National Laboratory. Martin Marietta Energy Systems, Inc. Oak Ridge, TN.

McKinley, C. R. and W. J. Lowe. 1986. Juvenile-mature correlations. Advanced Generation Breeding of Forest Trees. So. Coop. Series Bul. No. 309. Louis. Agric. Exper. Sta. p.11-16.

North Carolina State University-Industry Cooperative Tree Improvement Program. 1979. Twenty-third Annual Report. School of Forest Resources. North Carolina State University. Raleigh, NC.

Robinson, J. F., J. P. van Buijtenen, and E. M. Long. 1984. Traits measured on seedlings can be used to select for later volume of loblolly pine. So. Jour. of Appl. For. 8(1):59-64.

Sokal, R. R. and F. J. Rolf. 1969. Biometry. W. H. Freeman and Company. San Francisco. p.494-548.

ISOZYME HETEROZYGOSITY, PHENOTYPIC FAMILY STABILITY, AND INTER-FAMILY COMPETITION AMONG SEEDLINGS OF LOBLOLLY PINE

G. A. Tuskan[1/] and A. E. Wiselogel[2/]

Abstract--Five open-pollinated families of loblolly pine were used to produce a seedling population, which was used to establish a competition diallel containing 20 family combinations. Each subject family was grown in combination with all other families at four subject family to competitor ratios (i.e., 16.67:83.33, 33.33:66.67, 83.33:16.67 and 100.0:0.00). The change in competitor genotype and competitor frequency significantly influenced subject tree growth. Twenty-four enzymes representing 41 isozyme locus were examined per mother-tree and 16 enzymes representing 19 isozyme loci were examined in the progeny of each mother-tree. Percent heterozygosity per mother-tree, mean percent heterozygosity per mother-tree family and percent heterozygosity per isozyme loci per mother-tree family were correlated to six-month-old seedling measurements obtained from the competition diallel to test the relationship between heterozygosity, fitness and variability. Twelve correlations with individual isozyme heterozygosity were significant at the 0.05 level (four more than expected by chance). Relationships between isozyme heterozygosity, phenotypic stability and competition are discussed.

Additional keywords: horizontal starch gel electrophoresis, genotype x environment interactions, balancing selection, *Pinus taeda*.

Competition is a biological interaction that occurs when the combined demands of all individuals within a given area exceeds the supply of necessary resources. This definition implies that competitors, within a given environment, act as biological agents limiting each neighboring individual's (i.e. the subject tree) growth. Competition can be viewed as a genotype by genotype interaction nested within the genotype by environment interaction from the equation:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{GE}.$$

Where, the genotype by environmental interactions is partitioned into genotype by climatic and edaphic factors and into genotype by biological factors. The biological factors include both interspecific and intraspecific interactions. The genotype by biological factors interaction, in particular,

---

1/ Assistant Professor, Department of Horticulture and Forestry, North Dakota State University, Fargo, North Dakota.

2/ Research Associate, Forest Science Department, Texas A&M University, College Station, Texas.

the intraspecific interactions, can be intentionally manipulated so that within a single physical environment numerous biological environments are created through combinations of various genotypes. This paper is concerned with the intentional manipulation of intraspecific, inter-family interactions.

Forest trees, unlike most domesticated crops, have to be adapted to a wide range of environments across space and time. Long generation intervals, wide geographic ranges, yearly climatic changes and successional changes all contribute to the environment of a single tree. Phenotypic stability in genetically improved material is a desirable trait. Phenotypic stability is defined as a consistency of performance, as measured by "yield", across a range of environmental conditions. Phenotypic (yield) stability has been important to corn and wheat breeders, leading to the development of double cross hybrids, bulk populations and pure line mixtures. Jones (1985) states that it is the consistency in yield across many locations and not the individual superiority at any one location that led to the acceptance of double cross hybrids over single cross hybrids. Phenotypic stability can be achieved through 1) phenotypic plasicity, 2) individual buffering relying on heterozygosity or 3) population buffering relying on genotypic mixtures. Creation of variable environments through competitive interactions, and subsequent testing of specific genotypes may allow for the selection of phenotypic stability in long-lived forest species.

Hamrick et al. (1981) has reported that trees contain significantly more isozyme variability and heterozygosity than most herbaceous plants. Strauss and Libby (1984) have suggested that balancing selection in a fluctuating environment may favor heterozygotes and thus may act to maintain genetic variability in forest populations. Ledig et al. (1983) described three relationships between heterozygosity and forest tree ontogeny. They were, with increased heterozygosity, 1) fitness should increase (as measured by growth), 2) phenotypic variability (as measured by the coefficient of variability) should decrease or phenotypic stability increase and 3) longevity should increase.

Working with knobcone pine (Pinus attenuata Lemm.), Strauss and Libby (1984) reported a weak but significant relationship between heterozygosity and growth in crossbred progeny. This was particularly true in situations of fluctuating climatic conditions. Strauss and Libby (1984) also reported that a few polymorphic loci were responsible for the relationship between growth and heterozygosity, though no single locus could solely explain variation in growth. Likewise, Ledig et al. (1983) reported finding a few significant but inconsistent relationships between single locus heterozygosity and growth, and no significant relationships between heterozygosity and the coefficient of variability. Mitton et al. (1981) reported inconclusive results in the association of heterozygosity and growth in ponderosa pine (Pinus ponderosa Law.), lodgepole pine (Pinus contorta Dougl.) and aspen (Populus tremuloides Michx.). Heterozygosity was positively associated with growth in only one of the three species. Associations between heterozygosity and variability were inconsistent, being positive in two species and negative in one. Rajora (1986) found positive and negative correlations between heterozygosity and both rust resistance and growth variables in Populus hybrids.

Utilizing information from a seedling competition diallel study, this paper considers the relationship between isozyme heterozygosity and seedling height and shoot dry weight as expressed under various levels of inter-family competition.

MATERIALS AND METHODS

Competition Diallel

Seedlings from five open-pollinated families of loblolly pine (Pinus taeda L.) were used to establish a competition diallel containing 20 family combinations. The five families were S6PT6, S6PT3, S2PT10, S3PT7 and S4PT6. Each family was grown in combination with all other families as both a competitor and a subject family. Competitor frequency was varied in all combinations. The subject family to competitor family frequencies were 100.0:0.00, 83.33:16.67, 33.33:66.67 and 16.67:83.33. Competitor frequency levels per subject family/competitor combinations were replicated four times within a single greenhouse bench. Within each plot the competitor seedlings were four centimeters from the subject seedlings and the subject seedlings were 10 centimeters apart (Fig 1). A single border row surrounded the entire experiment. See Tuskan (1984) and Tuskan and van Buijtenen (1986a) for further details.

After 166 days the central subject seedlings were destructively sampled. Seedling height (mm) and shoot dry weight (mg) were recorded. Competitor genotypes had begun to influence subject seedling height at 72 days, and after 166 days mean subject family growth varied significantly ($\alpha \leq 0.05$) by competitor families (Tuskan 1984, Tuskan and van Buijtenen 1986a). Subject families and competitor families were classified as to their competitive ability and competitive influence, respectively.

Competitive effects for each family combination at each competitor frequency level were also calculated for subject seedling height and shoot dry weight. Competitive effects for a binary family combination were calculated as the sum of the mixed plot yields divided by the sum of the pure plot yields. Within a population, the mean competitive effect is expected to equal one (van Buijtenen and Tuskan 1986).

Starch Gel Electrophoresis

Standard horizontal starch gel electrophoresis procedures (Conkle et al. 1982, Adams and Joly 1980, Jech and Wheeler 1984, and Cheliak and Pitel 1984) were followed using three buffer systems. Table 1 contains a list of enzymes, enzyme abbreviations and buffer systems used in this study. Megagametophytic tissue (1N) was used to characterize the maternal genotype and embryonic tissue (2N) was used to characterize the progeny. Twenty-four enzymes representing 41 isozyme loci were scored for each mother-tree (i.e. acid phosphatase (ACP), aconitase (ACO), adenylate kinase (AK), aldolase (ALD), catalase (CAT), diaphorase (DIA), fumerase (FUM), glucose-6-P-dehydrogenase (G6PD), glucose dehydrogenase (GLUDH), glutamate dehydrogenase (GDH), glutamic oxyalocecetic transaminase (GOT), glyceraldehyde dehydrogenase (GLYDH), hexokinase (HK), isocitric dehydrogenase (IDH),

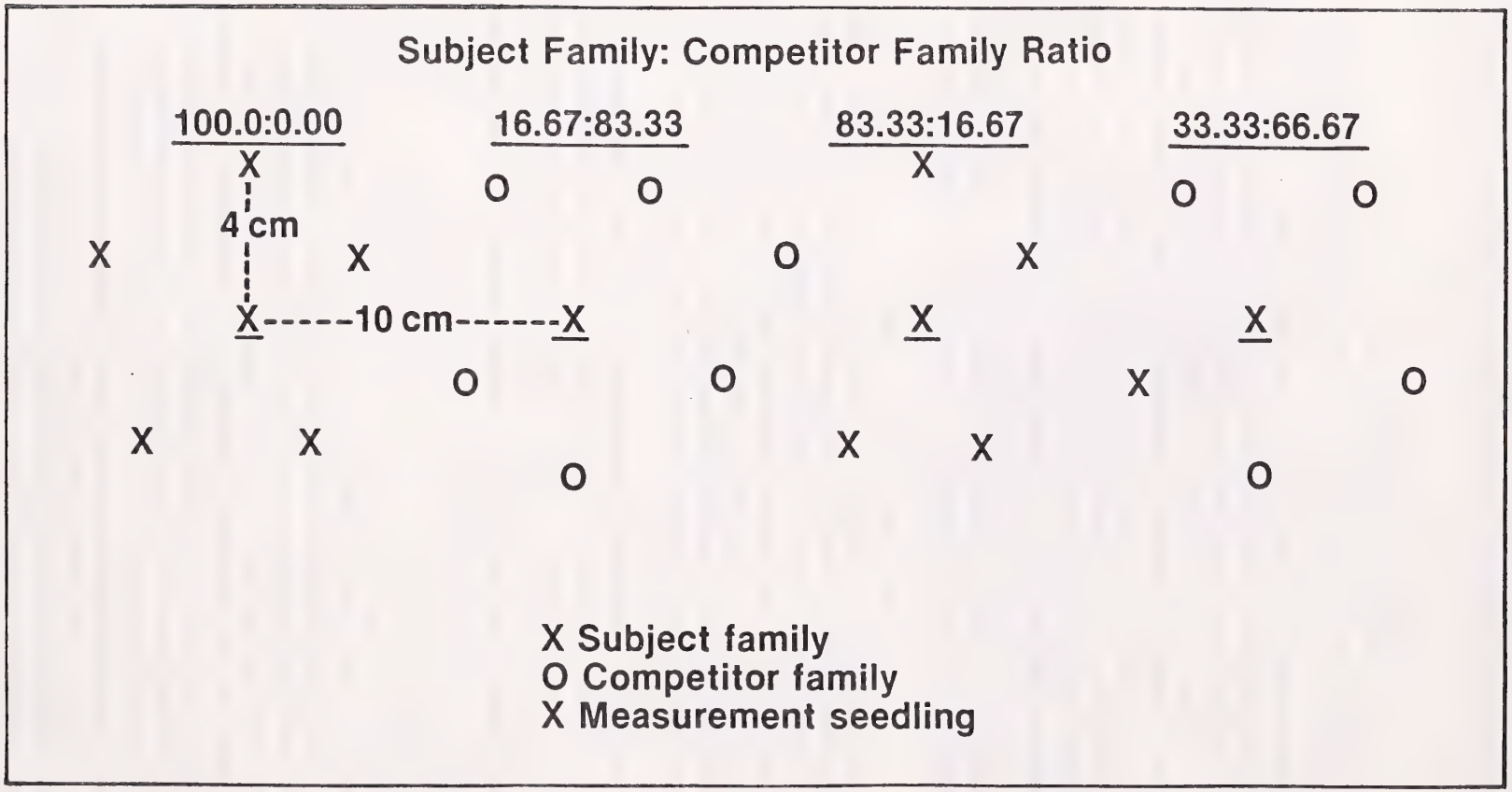


Figure 1. Representation of a single, randomized plot.

TABLE 1. ISOZYME DESCRIPTIONS INCLUDING ENZYME COMMISSION NUMBERS, BUFFER SYSTEM AND TISSUE TYPE.

Enzyme (Abbreviation)	E.C. Number	Buffer[1/] System	Megagametophyte Locus Number	Megagametophyte Number of Alleles	Embryo Locus Number
Acid Phosphatase (ACP)	3.1.3.2	M	1	2	1
			2	2	
Aconitase (ACO)	4.2.1.3	M	1	3	1
Adenylate Kinase (AK)	2.7.4.1	H	1	1	
			2	2	
			3	1	
Aldolase (ALD)	4.1.2.13	H	1	1	
			2	1	2
Catalase (CAT)	1.11.1.6	L	1	1	
Diaphorase (DIA)	1.6.4.3	M	1	1	1
Fumerase (FUM)	4.2.1.2	H	1	1	
Glucose-6-P-Dehydrogenase (G6PD)	1.1.1.49	M	1	2	1
Glucose Dehydrogenase (GLUDH)	1.1.1.47	H	1	1	
Glutamate Dehydrogenase (GDH)	1.4.1.3	L	1	3	
Glutamic Oxyaloacetic Transaminase (GOT)	2.6.1.1	L	1	2	
			2	2	
			3	2	
Glyceraldehyde Dehydrogenase (GLYDH)	1.1.1.29	H	1	2	1
Hexokinase (HK)	2.7.1.1	H	1	2	1
Isocitric Dehydrogenase (IDH)	1.1.1.42	M	1	1	1
Leucine Aminopeptidase (LAP)	3.4.11.1	L	1	1	
			2	2	
			3	2	3
Malate Dehydrogenase (MDH)	1.1.1.37	M	1	2	
			2	2	2
			3	2	3
			4	1	
Malic Enzyme (ME)	1.1.1.40	M	1	2	1
Menadione Reductase (MNR)	1.6.99.2	H	1	1	
			2	2	
			3	1	
6-Phosphogluconate Dehydrogenase (6PGD)	1.1.1.44	M	1	2	1
			2	2	2
Phosphoglucomutase (PGM)	2.7.5.1	H	1	2	1
			2	1	
Phosphoglucose Isomerase (PGI)	5.3.1.9	L	1	2	1
			2	2	2
Phosphomannose Isomerase (PMI)	5.3.1.8	L	1	3	1
Shikimate Dehydrogenase (SKDH)	1.1.1.25	M	1	3	1
Uridine Diphosphoglucose Pyrophosphorylase (UGP)	2.7.7.9	M	1	1	
			2	1	

1 M - Morpholine-citrate, pH = 6.1, Clayton and Tretiak (1972).
L - Lithium-borate, pH = 8.3, Ridgeway et al. (1970).
H - Histidine-citrate, pH = 7.0, Cheliak and Pitel (1984).

leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), menadione reductase (MNR), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), phosphomannose isomerase (PMI), shikimate dehydrogenase (SKDH) and uridine diphosphosphoglucose pyrophosphoglase (UGP)), and 16 enzymes representing 19 isozymes loci were scored for 40 progeny of each mother-tree (i.e. ACP, ACO, ALD, DIA, G6PD, GLYDH, HK, IDH, LAP, MDH, ME, 6PGD, PGM, PGI, PMI and SKDH). Percent heterozygosity per mother-tree, mean percent heterozygosity per mother-tree family and percent heterozygosity per isozyme loci per mother-tree family were determined for each family in the competition diallel.

Data Analysis

Estimates of heterozygosity were correlated with indicators of fitness and variability using Pearson's correlation coefficient. It was assumed that variables reflecting growth were related to fitness, in that larger trees which suppress or eliminate competitors, or trees which co-exist and complement their competitors would survive and reproduce at a higher frequency. Height and shoot dry weight per mother-tree family were used as indicators of growth while mean competitive effect for height and shoot dry weight per mother-tree family were used as indicators of competitiveness. The coefficients of variability for all fitness variables were used in correlations as inverse indicators of phenotypic stability. Subject family means and coefficients of variability were estimated across competitor genotypes, competitor frequencies and replications, totaling 64 seedlings per estimate.

RESULTS AND DISCUSSION

Isozyme Variability

Sixty-two percent of the isozyme loci were polymorphic in the mother-tree population and 89 percent were polymorphic in the progeny; the average number of alleles per locus was 1.71 (Table 1). The difference between the percent polymorphic loci for the mother-trees and percent polymorphic loci for the progeny reflects sampling error. A greater number of monomorphic loci were scored for the mother-trees. The percent of polymorphic loci for the mother-tree population and the progeny are identical when considering the same loci. The estimates for percent polymorphic loci and number of alleles per locus are consistent with results reported by Hamrick *et al.* (1981) for other coniferous species. The 16 monomorphic loci in the mother-tree population and the two monomorphic in the progeny were not used in the remainder of the study.

The mean percent heterozygosity did not vary greatly between the mother-trees and their respective progeny (Table 2). The greatest change in heterozygosity occurred with mother-tree S6PT3. The percent heterozygosity in the progeny of S6PT3, and progeny of all other mother-trees, was due to the number of heterozygous loci in the mother-tree and the sampling of gametes in the pollen cloud. The probability of the progeny being heterozygous at a locus which is heterozygous in the mother-tree, regardless of the allelic frequency in the pollen cloud, is 50 percent. If a mother-tree is homozygous at a locus then the probability that the progeny will be heterozygous at that

TABLE 2. PERCENT HETEROZYGOSITY FOR INDIVIDUAL MOTHER-TREES, MOTHER-TREE FAMILIES AND INDIVIDUAL ISOZYMES WITHIN MOTHER-TREE FAMILIES.

	Mother-Tree Family				
	S6PT6	S6PT3	S2PT10	S3PT7	S4PT6
% Heterozygosity per Mother-tree	17.1	43.1	26.8	17.1	19.5
Mean % Heterozygosity per Mother-tree family	19.0	17.9	24.5	22.9	19.5
% Heterozygosity per Mother-tree family for:					
Acp1	0.0	0.0	0.0	0.0	0.0
Aco	29.3	42.1	50.0	31.8	28.9
Ald2	24.4	11.9	10.0	22.7	26.3
Dia	11.1	21.4	43.3	8.3	5.3
Glydh	43.9	57.1	60.0	81.8	36.8
G6pd	14.6	2.4	20.0	59.1	42.1
Hk	0.0	21.7	26.7	16.7	40.0
Idh	0.0	2.4	0.0	0.0	0.0
Lap3	14.6	7.1	10.0	4.5	2.6
Mdh2	2.4	2.4	13.3	54.5	0.0
Mdh3	48.8	21.4	36.7	45.5	31.5
Me	24.4	52.4	36.7	40.9	42.1
6Pgd1	39.0	40.5	36.7	50.0	71.1
6Pgd2	2.4	14.3	23.3	13.6	2.6
Pgi1	0.0	0.0	0.0	0.0	0.0
Pgi2	19.5	0.0	33.3	4.5	34.8
Pgm1	39.0	4.8	10.0	0.0	0.0
Pmi	0.0	0.0	0.0	16.7	13.0
Skdh	7.3	52.6	25.0	9.1	0.0

locus depends upon the frequency of the alternative alleles. Thus, a mother-tree which is homozygous for rare alleles at several loci will have a greater chance of producing a higher percentage of heterozygous progeny than a mother-tree which is heterozygous at the same loci. Examples of mother-trees which are heterozygous for rare alleles are S6PT6 at Pgm1, S6PT3 at Skdh and S3PT7 at Mdh2 (Table 2). In all of these cases the mother-trees produced heterozygous progeny approximately 50 percent of the time.

Indicators of Fitness and Variability

At the end of six months mother-tree families had significantly ($\alpha \leq 0.05$) different mean heights. Mean heights ranged from 162 mm for S6PT3 to 186 mm for S2PT10 (Table 3). The coefficients of variability for height also varied from 4.75 for S3PT7 to 14.81 for S6PT6. The mean competitive effects for height ranged from .968 for S4PT6 to 1.013 for S6PT6. Mean competitive effects below 1.00 indicate that mixtures containing that particular mother-tree family performed poorer on the average than the sum of the pure plots. Conversely values greater than 1.00 indicate that mixtures containing that particular family did better than the sum of the pure plots.

Values for mean shoot dry weight ranged from 318 mg for S6PT6 to 394 mg for S4PT6 (Table 3). Mean competitive effects for shoot dry weight ranged from 0.878 for S6PT3 to 1.007 for S3PT7. S6PT6 had the highest coefficient of variability for shoot dry weight and for the competitive effect for shoot dry weight, while S3PT7 had the lowest coefficient of variability for both variables.

Subject family performance was significantly influenced by competitor genotype (Tuskan 1984, Tuskan and van Buijtenen 1986a, 1986b). Competitor frequency also influenced subject family performance (Table 4). S2PT10 ranged from having one of the poorest heights in the study, (137 mm with S4PT6 at a 16.67 : 83.33 competitor to subject family ratio) to having the best height in the study (230 mm with S6PT6 at a 16.67:83.33 competitor to subject family ratio).

TABLE 4. SEEDLING HEIGHT FOR SUBJECT FAMILY S2PT10 GROWN WITH ALL COMPETITOR GENOTYPES AND FREQUENCIES.

Competitor Frequency	Competitor Genotype			
	S6PT6	S6PT3	S3PT7	S4PT6
	mm			
0.00	190	190	190	190
16.67	230	180	185	137
66.67	195	171	197	174
83.33	214	169	189	192

Correlations between heterozygosity and indicators of fitness.

Assuming heterozygosity increases fitness and phenotypic stability, then correlations between heterozygosity and indicators of fitness should be

TABLE 3. MEAN HEIGHT, SHOOT DRY WEIGHT AND COMPETITIVE EFFECTS FOR HEIGHT AND SHOOT DRY WEIGHT OBTAINED FROM 6-MONTH-OLD LOBLOLLY PINE SEEDLINGS GROWN IN A COMPETITION DIALLEL.

Mother-tree Family	Height at 166 days(mm)		Shoot Dry Weight (mg)		Competitive Effect[1/] for Height		Competitive Effect[1/] for Shoot Dry Weight	
	Mean[2/]	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
S6PT6	173 A	14.81	318 A	32.56	1.013	9.60	0.991	20.39
S6PT3	162 B	13.13	322 A	19.00	0.979	6.93	0.878	17.06
S2PT10	186 A	12.61	338 A	25.84	1.000	9.32	0.995	17.99
S3PT7	171 A	4.75	343 A	18.56	0.993	3.94	1.007	8.71
S4PT6	176 A	5.70	394 A	19.96	0.968	5.57	0.893	12.79

1/ Competitive Effect = mixture yields divided by pure-stand yields; i.e., $(Y_{ij} + Y_{ji})/(Y_{ii} + Y_{jj})$.

2/ Means connected by similar letters are not significantly different at $\alpha \leq 0.05$ as determined by SNK's multiple range test.

positive, while correlations between heterozygosity and indicators of variability should be negative. Though none of the correlations using percent heterozygosity per mother-tree or mean percent heterozygosity per mother-tree family were significant ($\alpha \leq 0.05$), the correlations between mean percent heterozygosity per mother-tree family and all indicators of fitness and stability reflected the hypothesized relationships (Table 5). The correlations involving height, shoot dry weight and the competitive effects for height and shoot dry weight were positive, while correlations involving the coefficients of variability were all negative.

Correlations involving estimates of heterozygosity for individual isozymes revealed 12 significant ($\alpha \leq 0.05$) relationships; three with indicators of fitness and nine with indicators of variability (Table 5). The individual isozymes involved were G6pd, Lap3, Mdh3, Me, 6Pgd1, Pgm and Pmi. Eight of the significant correlations occurred with three of the 16 isozymes; i.e., G6pd, Lap3 and Pmi. Nine of the significant correlations displayed the predicted relationships, particularly those involving G6pd and Pmi. The three significant correlations involving indicators of fitness were all positive, as predicted, and six of the nine significant correlations involving indicators of variability were negative.

Competitor genotypes and frequencies were used in this study to rank mother-tree family performance in various competitor environments. The extreme responses are: 1) a U-shaped curve, indicating superior performance in a few environments and inferior performance in others; and 2) a bell-shaped curve, indicating average performance across environments (Allard 1961). Based on the relationship between heterozygosity and phenotypic stability we expected U-shaped curves for more homozygous progeny and bell-shaped for more heterozygous progeny. Mother-tree family S3PT7 displayed a bell-shaped curve with average performance across competitive environments and was the family with the highest levels of heterozygosity for G6pd and Pmi (Fig 2). Conversely, S6PT6 had a U-shaped curve with superior performance in some competitive environments and inferior performance in others. S6PT6 had one of the lowest percent heterozygosities for G6pd and Pmi. The remaining mother-tree families were intermediate in curve shape and percent heterozygosity. Similar curves and results can be obtained for shoot dry weight, and competitive effects for height and shoot dry weight.

Isozyme heterozygosity may be related to fitness and phenotypic stability by three means. First, isozyme heterozygosity may be an indicator of inbreeding depression. Ledig *et al.* (1983) suggests that deleterious genes are expressed as a result of ancestral mating, and therefore outcrossing as indicated by heterozygosity is advantageous. It is unlikely though that inbreeding occurred during the creation of the progeny used in this study since all mother-trees are first generation selections contained in a clone bank. Second, individual allelic differences in the enzymes themselves may convey an advantage to heterozygous individuals (Fincham 1972). Electrophoretic techniques stain for enzymes involved in major metabolic pathways, and it is improbable that fitness is directly and solely related to such genes. Finally, heterozygosity may be related to fitness through linkage of isozyme variants and genes directly related to survival and reproduction. Stuber *et al.* (1982) reported, that because of linkage, isozyme patterns in

TABLE 5. PEARSON'S CORRELATION COEFFICIENTS AMONG INDICATORS OF HETEROZYGOSITY, GROWTH AND VARIABILITY.

	Height at 166 days		Shoot Dry Weight		Competitive Effect for Height		Competitive Effect for Shoot Dry Weight	
	Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
	r							
% Heterozygosity per mother-tree	-.42	.43	-.35	-.34	-.34	.13	-.61	.31
Mean % Heterozygosity per mother-tree family	.74	-.25	.06	-.01	.31	-.01	.71	-.28
% Heterozygosity per mother-tree family for:								
Aco	.34	.44	-.35	-.04	.09	.42	.03	.35
Ald	-.13	-.51	.47	.08	-.07	-.38	.06	-.37
Dia	.53	.52	-.36	.20	.28	.59	.20	.47
Glydh	-.12	-.30	-.34	-.40	.20	-.44	.50	-.53
G6pd	.18	-.93*	.59	-.42	-.20	-.76	.34	-.89*
Hk	.27	-.53	.81	-.64	-.84	-.39	-.55	-.41
Idh	-.72	.35	-.39	-.39	-.77	.03	-.67	.20
Lap3	.17	.86	-.75	.92*	.87*	.88*	.45	.85
Mdh2	-.02	-.58	-.06	-.37	.17	-.61	.60	-.74
Mdh3	.32	-.07	-.18	.58	.76	.12	.88*	-.05
Me	-.47	-.31	.21	-.89*	-.80	-.56	-.69	-.41
6Pgd1	.01	-.78	.94*	-.49	-.74	-.64	-.44	-.61
6Pgd2	.32	.14	-.32	-.22	.13	.12	.27	-.01
Pgi2	.85	-.03	.57	.36	-.02	.37	.08	.21
Pgm	.07	.72	-.57	.95*	.79	.76	.38	.76
Pmi	-.06	-.98*	.66	-.60	-.43	-.90*	.06	-.95*
Skdh	-.42	.51	-.55	-.25	-.12	.19	-.41	.33

* - indicates a significant correlation at $\alpha \leq 0.05$.

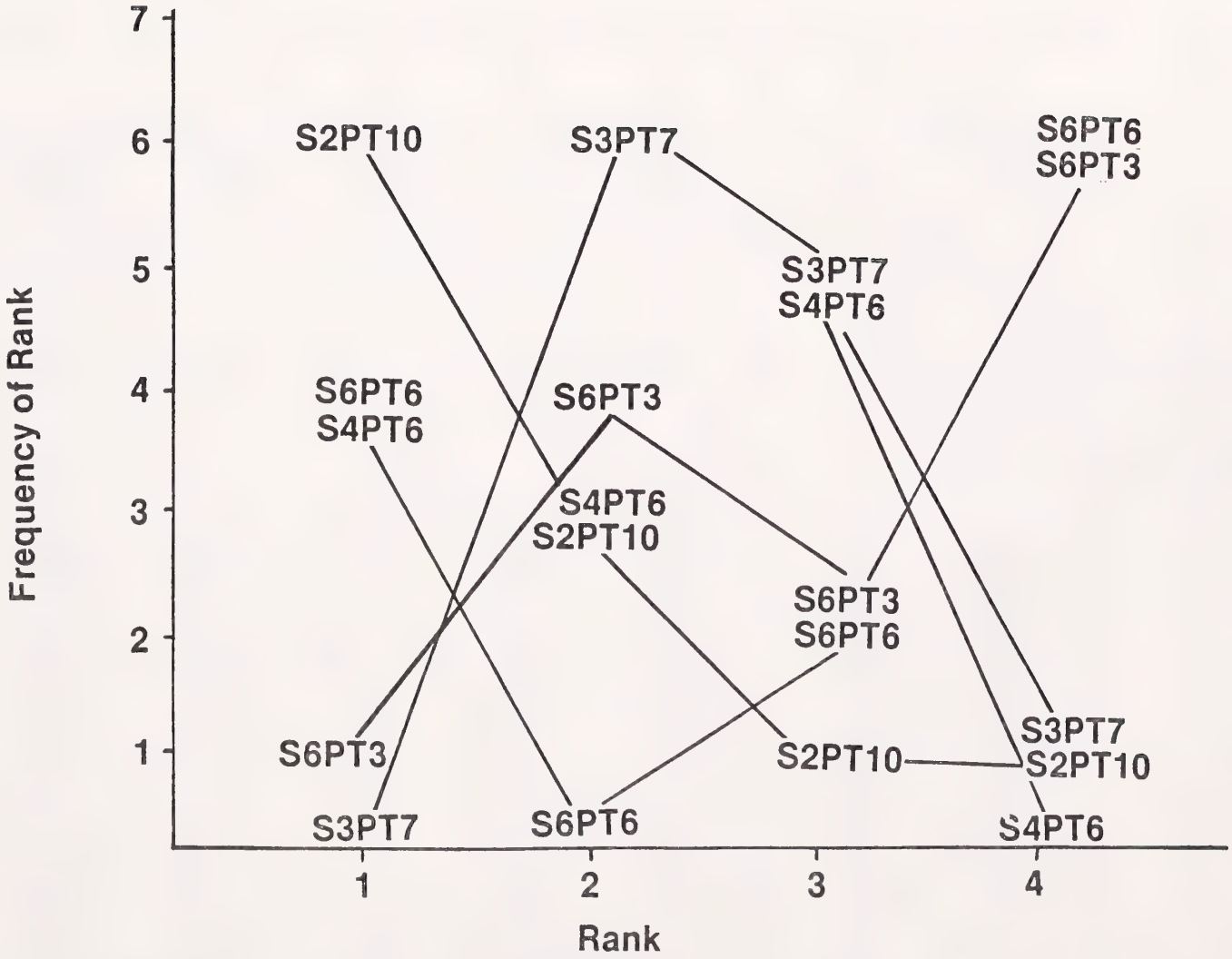


Figure 2. Frequency with which mother-tree families ranked first through fourth for seedling height in 15 competitor genotype-competitor frequency environments.

selected populations of corn could be used to improve unselected populations by breeding solely for the isozyme pattern displayed in the improved population.

It is likely that heterozygosity in genes for growth habit would be important in situations where competition is occurring, particularly when competitive effects are greater than one. For seedling mixtures to perform better than the mean of the pure plots limited resources would have to be utilized more efficiently within the mixed plots. Several plant growth habits--stratified seedling architecture, nutritional compensation and asychronous growth, have been used to explain superior performance of mixtures (Trenbath 1974), and could possibly be the result of heterozygosity in a mother-tree family growing in competition with a genetically dissimilar family.

SUMMARY

The long life expectancy of most forest trees creates a situation where genotypes within a population are exposed to continuously fluctuating environmental conditions. Climatic variability and changing competitive circumstances may act to maintain genetic variability through balanced selection over the life of a forest stand. Several authors have reported that trees contain significantly more isozyme variability than other plant species and that heterozygosity may convey some adaptive advantage in fluctuating environments. A competition diallel was used to create several different competitive environments in which the relationship between heterozygosity, fitness and phenotypic stability could be tested for five mother-tree families of loblolly pine. Significant relationships occurred 12 times, mainly involving isozyme heterozygosity in G6pd and Pmi and the coefficients of variability for height, shoot dry weight and the competitive effects for height and shoot dry weight. Correlations indicated that as heterozygosity for G6pd and Pmi increased phenotypic stability increased. A weak, positive relationship was also expressed between heterozygosity and fitness as measured by shoot dry weight and the competitive effects for height and shoot dry weight.

LITERATURE CITED

Adams, W.T. and R.J. Joly. 1980. Genetics of allozyme variants in loblolly pine. J. Hered. 71:33-40.

Allard, R.W. 1961. Relationship between genetic diversity and consistency of performance in different environments. Crop Sci. 1(2):127-133.

Cheliak, W.M. and J.A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Can. For. Serv. Information Report PI-X-42. 49 pp.

Clayton, J.W. and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish Res. Board Can. 29:1169-1172.

Conkle, M.T., P.D. Hodgskiss, L.B. Nunnally and S.C. Hunter. 1982. Starch gel electrophoresis of conifer seeds: a laboratory manual. USFS Gen. Tech. Report PSW-64. 18 pp.

Fincham, J.R.S. 1972. Heterozygous advantage as a likely general basis for enzyme polymorphism. Heredity 28(3):387-391.

Hamrick, J.L., J.B. Mitton and Y.B. Linhart. 1981. Levels of genetic variation in trees: Influence of life history characteristics. In USFS Gen. Tech. Rep. PSW-48. p. 35-41.

Jech, K.S. and N.C. Wheeler. 1984. Laboratory manual for horizontal starch gel electrophoresis. Weyerhauser Tech. Report 050-3210/6. 61 pp.

Jones, D.F. 1958. Heterosis and homeostasis in evolution and applied genetics. Am. Nat. 42:321-328.

Ledig, F.T., R.P. Guries and B.A. Bonefeld. 1983. The relationship of growth to heterozygosity in pitch pine. Evolution 37(6):1227-1238.

Mitton, J.B., P. Knowles, K.B. Sturgeon, Y.B. Linhart and M. Davis. 1981. Associations between heterozygosity and growth rate variables in three western forest trees. In USFS Gen. Tech. Rep. PSW-48. p. 27-34.

Rajora, O.P. 1986. Association of allozymes with growth parameters and Melampsora rust resistance in full-sib families of Populus deltoides Marsh. crossed with P. deltoides, P. nigra and P. maximowiczii Henry. Proc. 9th North American Forest Biology Workshop, Stillwater, OK. p. 219.

Ridgeway, G.J., S.W. Sherburne and R.D. Lewis. 1970. Polymorphisms in the esterases of Atlantic herring. Trans. Am. Fisheries Soc. 99:147-151.

Strauss, S.H. and W.J. Libby. 1984. Use of isozyme variation to predict productivity and stability of biomass crops. Final Report to the University of California Appropriate Technology Program. 22 pp.

Stuber, C.W., M.M. Goodman and R.H. Moll. 1982. Selection at allozyme loci in maize. Crop Sci. 22(4):737-740.

Trenbath, B.R. 1974. Biomass productivity in mixtures. Adv. Agron. 26:177-210.

Tuskan, G.A. 1984. An investigation of inter-family competition in loblolly pine. Ph.D. Dissertation. Texas A&M University, College Station, TX. 97 pp.

Tuskan, G.A. and J.P. van Buijtenen. 1986a. Inherent differences in family response to inter-family competition in loblolly pine. Silvae Genetica 35(2-3):112-118.

Tuskan, G.A. and J.P. van Buijtenen. 1986b. The detection of inter-family competition among seedlings of loblolly pine. Proc. 9th North American Forest Biology Workshop, Stillwater, OK. p. 208-217.

van Buijtenen, J.P. and G.A. Tuskan. 1986. Design of control pollinated progeny and clonal tests. In Workshop of the Genetics and Breeding of Southern Forest Trees. June 25-26, 1986. Univ. of Florida, Gainsville, FL.

RESULTS OF MICROPULPING WOOD SAMPLES OF SEVEN PINE HYBRID FAMILIES IN ZULULAND

J.A. Wright[1]

Abstract.--Micropulping of increment cores was used to assess families of *Pinus elliottii* Engelm., *P. caribaea* var. *bahamensis* Barr. and Golf. and hybrids of *P. elliottii* crossed with *P. caribaea* var. *hondurensis* Barr. and Golf. and *P. cubensis* Griseb. growing in Zululand, South Africa. Families were significantly different for screened pulp yield(%), total pulp yield(%), stretch(%), tear index, bulk specific and tensile energy absorbed. The high tear index and superior volume production of two families of the hybrid between *P. elliottii* and *P. caribaea* var. *hondurensis* relative to *P. elliottii* indicates the potential of these hybrids for sites similar to the one evaluated here.

The hybrid between *Pinus elliottii* Engelm. and *P. caribaea* var. *hondurensis* Barr. and Golf. has certain advantages relative to the parent species for a range of soil and climatic conditions. On swampy sites in Queensland, Australia, the hybrid has exhibited better growth than *P. elliottii* as well as superior stem quality and wind resistance when compared to *P. caribaea* var. *hondurensis* (Slee, 1969;1971). It was decided by the Queensland Forestry Department in 1985, to use seedlings of the hybrid for routine planting on swampy sites (Nikles *et al.*, 1987).

In South Africa, the development of this hybrid was undertaken to combine the rapid growth of *P. caribaea* var. *hondurensis* with the superior stem form and higher wood density of *P. elliottii*. A trial of numerous pine species and hybrids was established by the South African Forestry Research Institute (SAFRI) on a uniform site in 1970, at the Futululu Forestry Research Station, Zululand. The trial was measured in 1984 at an age of 13.5 years and the four families of *P. elliottii* crossed with *P. caribaea* var. *hondurensis* were found to have twice the mean annual increment (M.A.I.) of *P. elliottii* families (van der Sijde and Roelofsen, 1986).

[1]Graduate Student, Oxford Forestry Institute, Oxford, England. Current address is Sappi Forests, PO Box 75, Pietermaritzburg 3200, South Africa.

The wood quality of these hybrids is of considerable interest in South Africa since at least one industrial organization will purchase only small quantities of P. caribaea Mor. due to the inferior paper resulting from wood of that species. A comparison of the wood, pulp and papermaking traits of P. elliottii and hybrid families from the Futululu trial was undertaken by Du Plooy (1984). He concluded that high density trees of the hybrid families were comparable to trees from families of P. elliottii for pulp yield and all of the paper strength traits except tearing strength. That study did not maintain family or tree identity and hence no superior genotypes with regard to pulp and papermaking traits could be identified.

METHODS

The families chosen for micropulping in the SAFRI trial at Futululu were selected for volume at the 1984 assessment when they were 13.5 years old (Table 1). Represented in this study are four families of the P. elliottii by P. caribaea var. hondurensis hybrid as well as single families of P. elliottii, P. caribaea var. bahamensis Barr. and Golf. and the hybrid between P. elliottii and P. cubensis Griseb. The trial is at a latitude of 28°21'S and an altitude of 70m. The trial design was a randomized complete block with four replications using row plots of ten trees at a square spacing of 2.7m. The trees had been pruned to 5m in three stages and a 40% silvicultural thinning was undertaken at the age of 10 years by the removal of four trees per row (van der Sijde and Roelofsen, 1986). In each replication, the largest diameter tree in the selected families was sampled by removing seven increment cores of 12mm diameter at about breast height (1.3m). This sampling took place in June, 1985, at the age of 14.75 years and a total of 28 trees were thus sampled for micropulping at Sappi's Enstra research centre[1]. The micropulping technique has been previously used by Sappi to assess trees of fast-growing tropical pines in South Africa (Wright, 1987a,b).

Table 1.--Sample identification for micropulping.

Family	Source (sample numbers)	Volume/tree at 13.5 years (m^3)	M.A.I. at 13.5 years(m^3/ha)
E1XC5	hybrid(95-98)	0.434	24.8
E503XC23	hybrid(99-102)	0.382	21.8
E38XC6	hybrid(87-90)	0.370	21.2
E1XC23	hybrid(91-94)	0.360	20.6
E1XCUB14	hybrid(83-86)	0.294	16.8
AND	P. caribaea var. bahamensis-Andros, Bahamas(79-82)	0.275	15.7
E49XM	poly-cross(75-78)	0.179	10.2

[1]PO Enstra 1561, South Africa

The increment cores were chipped into small segments and placed in a controlled temperature and humidity room where they were allowed to come to a moisture content of approximately 10.5%. A kraft cook was used and the cooking conditions are listed in Table 2. The pulp was beaten for 15,000 revolutions in a Lampen beater. Hand sheets were made using the Frank Sheet Machine and were conditioned to constant temperature and humidity prior to sheet testing. The micropulping procedures used at Enstra are similar to those developed by Mr. E.R. Palmer at the Tropical Development and Research Institute, London, and require a minimum of 65g of air dried wood.

The pulp and papermaking traits were analyzed using analysis of variance (ANOVA) with families as fixed effects. Sampling the largest diameter tree in each family was considered to be an observation rather than a replication and this source was a random effect. Differences between families and trees were compared at the 5% level using the Q statistic as described by Chew (1977). The amount of pulp from one tree of family E38XC6 was insufficent for beating in the Lampen beater and the degrees of freedom in the residual term have consequently been reduced for certain traits.

Table 2. Cooking conditions.

Maximum temperature (°C)	170
Time to 170°C (minutes)	110
Time at 170°C (minutes)	60
Liquor to oven dry wood ratio	5.4:1
Active alkali as Na_2S % of oven dry wood	22

RESULTS AND DISCUSSION

The results of the ANOVA are contained in Table 3. The most important kraft pulp and papermaking traits for Sappi are burst index, tear index and total pulp yield(%). Family means of these traits as well as M.A.I. values are presented in Figure 1. The values of these pulp and papermaking traits for individual trees are given in Figure 2 as are family means of M.A.I.

Families were significantly different ($p<0.05$) for screened pulp yield(%), total pulp yield(%), stretch(%), tear index, bulk specific and tensile energy absorbed (T.E.A.). The high probability (p) values for family differences in all of the traits except wetness and Canadian standard freeness (C.S.F.) would suggest superior families could be selected for papermaking properties. However, no individual family was found to be above the trial mean for all of the traits presented in Figure 1.

It is interesting that the families E1XCUB14 and E38XC6 rank first and last for burst index, respectively, but the ranking is reversed for tear index. This indicates the likely difficulty in attempting to improve these two traits simultaneously. It should

Table 3.--ANOVA of micropulping results from seven pine hybrid families in Zululand, South Africa.

Trait	Source	df	Mean square	(p value)
Kappa no.	Observations	3	12.56	
	Families	6	14.97	(p=0.074)
	Residual	18	6.36	
Screened yield(%)	Observations	3	18.17	
	Families	6	43.97	(p=0.009)
	Residual	18	10.65	
Reject yield(%)	Observations	3	7.84	
	Families	6	3.01	(p=0.207)
	Residual	18	1.89	
Total yield(%)	Observations	3	6.60	
	Families	6	54.03	(p=0.005)
	Residual	18	11.51	
Spent liquor(AA)	Observations	3	0.05	
	Families	6	1.69	(p=0.184)
	Residual	18	1.01	
Spent liquor(NA)	Observations	3	0.82	
	Families	6	2.26	(p=0.244)
	Residual	18	1.54	
Total solids(%)	Observations	3	0.04	
	Families	6	0.54	(p=0.150)
	Residual	18	0.29	
Wetness	Observations	3	12.79	
	Families	6	5.35	(p=0.851)
	Residual	17(1)	12.55	
C.S.F.	Observations	3	6.33×10^{3}	
	Families	6	1.36×10^{3}	(p=0.907)
	Residual	17(1)	4.01×10^{3}	
Stretch(%)	Observations	3	0.04	
	Families	6	0.37	(p<0.001)
	Residual	17(1)	0.05	
Breaking length	Observations	3	2.76×10^{5}	
	Families	6	6.29×10^{5}	(p=0.126)
	Residual	17(1)	3.18×10^{5}	
Burst index	Observations	3	0.17	
	Families	6	0.35	(p=0.132)
	Residual	17(1)	0.18	
Tear index	Observations	3	0.20	
	Families	6	3.12	(p=0.006)
	Residual	17(1)	0.67	
Bulk specific	Observations	3	1.14×10^{-3}	
	Families	6	7.58×10^{-3}	(p=0.003)
	Residual	17(1)	1.44×10^{-3}	
Porosity	Observations	3	2.23×10^{6}	
	Families	6	4.53×10^{6}	(p=0.129)
	Residual	17(1)	2.32×10^{6}	
T.E.A.	Observations	3	2.02×10^{3}	
	Families	6	5.98×10^{3}	(p=0.001)
	Residual	17(1)	9.34×10^{3}	

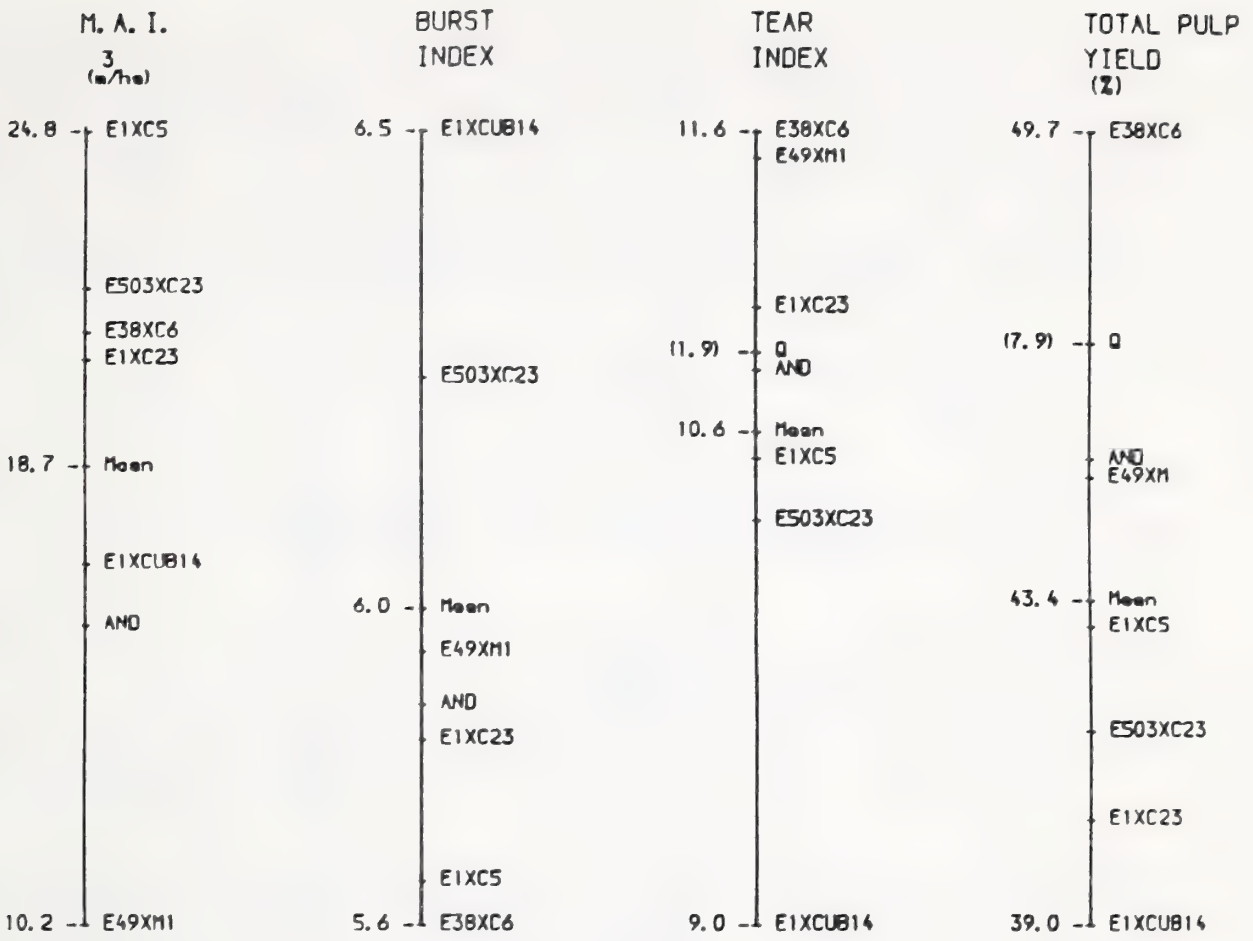
be noted, however, that family E38XC6 had almost twice the M.A.I. and was also superior for tear index and total pulp yield(%) to the P. elliottii family E49XM. The most commonly planted conifer in Zululand is currently P. elliottii. The high tear index and M.A.I. of the hybrid families E38XC6 and E1XC23 would suggest that they be included in future afforestation programs. There were no individual trees which were ranked above the mean for all of the papermaking traits presented in Figure 2. One tree from each of family E38XC6 and E1XC23 was ranked above the mean for tear index and total pulp yield(%) and was only slightly below the mean for burst index.

CONCLUSIONS

The use of gravimetric density may not be a satisfactory method of assessing wood quality since trees with high wood density may have reduced breaking length and burst index relative to trees with lower density (Barefoot et al., 1972;Burley and Palmer, 1979;Wright, 1987b). Micropulping can be used to sample individual trees non-destructively for their papermaking properties. The use of micropulping to determine superior phenotypes for pulp and papermaking traits is being used by two organizations (Sappi and Aracruz in Brazil) to indicate those trees which should be included in seed orchards and/or used for the production of vegetative propagules. For the pines, we do not have heritability values for the pulp and papermaking traits and these are urgently needed before the onset of breeding activities. However, it is expected that selection and silvicultural management can significantly increase the quantity and quality of the kraft paper produced from these plantations.

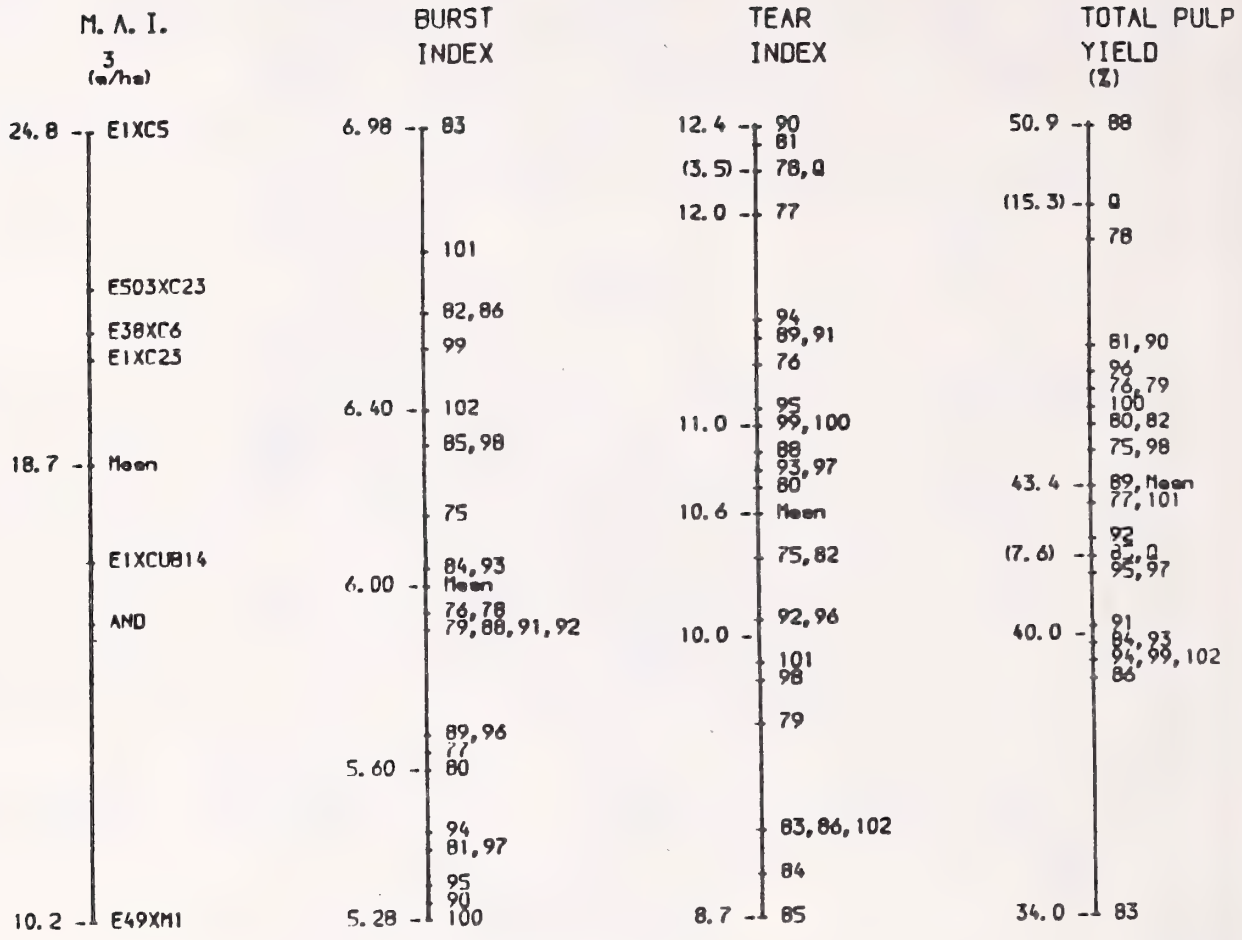
ACKNOWLEDGEMENTS

I would like to thank Mr. Dave Steyn at the Futululu Forestry Research Station and the South African Forestry Research Institute for their assistance in obtaining the wood samples. Drs. J. Burley and R.D. Barnes of the Oxford Forestry Institute kindly read the manuscript and made useful comments.



Source	df	MS (p value)		
Replications	3	0.17	0.20	6.60
Family	6	0.35 (p=0.132)	3.12 (p=0.006)	54.03 (p=0.005)
Residual	17 (11)	0.18	0.67	11.51
s.e. family		0.21	0.41	1.70

FIGURE 1. Analysis of variance, ranked means and the critical difference, Q (p=0.05), for M. A. I., burst index, tear index and total pulp yield (%) of seven pine families in Zululand, South Africa.



Source	df	MS (p value)		
Family	6	0.35 (p=0.119)	3.12 (p=0.003)	54.03 (p=0.003)
Residual	20 (1)	0.18	0.60	10.81
s.e. tree		0.42	0.77	3.29

FIGURE 2. Analysis of variance, individual tree means and the critical differenc Q (p=0.05), for M. A. I., burst index, tear index and total pulp yield (% of seven pine families in Zululand, South Africa.

LITERATURE CITED

Barefoot, A.C., R.G. Hitchings, E.H. Wilson and R.C. Kellison. (1972). Related aspects of the morphology of loblolly pine and papermaking. In:'Symposium on the effect of growth acceleration on the properties of wood'. Madison, Wisconsin, November, 1971. Forest Products Laboratory, Madison. K1-K33.

Burley, J. and E.R. Palmer. 1979. Pulp and wood densitometric properties of Pinus caribaea from Fiji. Occasional Paper No. 6, Commonwealth Forestry Institute, Oxford, 66 p.

Chew, V. 1977. Comparisons among treatmeant means in an analysis of variance. Agricultural Research Service, U.S.D.A., Washington, D.C., 64 p.

Du Plooy, A.B.J. 1984. Kraft pulp properties of 15-year old P. elliottii x P. caribaea var. hondurensis. Report C/Hout 315, Council for Scientific and Industrial Research, Pretoria, 22 p.

Nikles, D.G., P.C. Bowyer and R.L. Eisemann. 1987. Performance and potential of hybrids of slash and Honduras pines in the subtropics. In:'Simposio sobre silvicultura y mejoramiento genetico de especies forestales'. Buenos Aires, Argentina, April, 1987. Centro de Investigaciones y Experiencias Forestales, Buenos Aires. I:68-79.

Slee, M.U. 1969. Height and girth growth of slash-caribbean hybrids in south Queensland. In:'Proceedings of Second World Consultation on Forest Tree Breeding.' Washington, August, 1969. FAO, Rome. 1:139-152.

Slee, M.U. 1971. Windfirmness and stem straightness in hybrids of slash and caribbean pines. Australian Forest Research 5(3): 21-28.

van der Sijde, H.A. and J.W. Roelofsen. 1986. The potential of pine hybrids in South Africa. South African Forestry Journal 136:5-14.

Wright, J.A. 1987a. Results of micropulping wood samples of Pinus caribaea, P. elliottii, P. oocarpa and P. patula ssp. tecunumanii in the Eastern Transvaal and Zululand. In:'Simposio sobre silvicultura y mejoramiento genetico de especies forestales'. Buenos Aires, Argentina, April, 1987. Centro de Investigaciones y Experiencias Forestales, Buenos Aires. IV:247-256.

Wright, J.A. 1987b. Provenance variation in wood characteristics of Pinus caribaea Morelet and P. oocarpa Schiede. Unpublished D.Phil. thesis, Department of Plant Sciences, Oxford University, 153 p.

DIFFERENCES IN GROWTH RATE AND IN ACCELERATION OF GROWTH RATE AMONG LOBLOLLY PINE RANGEWIDE SEED SOURCES CONFIRMED

Fan H. Kung[1]

Abstract.--Loblolly pine (Pinus teada L.) from 15 rangewide seed sources were planted in 15 widely separated plantations. Height growths at 3, 5, 10, 15, 20 and 25 years of age indicated that differences in growth rate and in acceleration of growth rate existed and should be useful for selection. The average growth rate for the study was 20 percent, but for the best seed sources (No. 329 and NO. 317), it was 25 percent per year. While seed sources No. 305 and No. 319 had fast growing rates only before age 15, seed source 321 was a slow starter, but had the best growth rate in the later years. Selection from fast growth rate would result in simultaneous selection for slow "incremental relative rate of relative growth rate".

Additional Keywords: Pinus teada; growth curves, provenance testing

INTRODUCTION

When the size of a tree or a forest is plotted over its age, the curve so defined is commonly called the growth curve. The cumulative growth curves are usually sigmoid-shaped, and therefore can be recognized in three or four phases of growth development (Husch et al., 1972; Switzer and Shelton, 1981). The phenotypic variance, genetic variance and heritability related to these growing periods, has been revealed (Namkoong et al., 1972; Franklin, 1979). During the juvenile acceleration phase, a period of comparatively free growth, seedlings that are genetically superior in the non-competitive condition grow faster than those that are less well adapted. The result is that in the plantation, the genetic variance among populations is low, but increasing. On the other hand, the environmental error increases logarithmically, so there may be a juvenile genetic culmination in this phase as the acceleration of error variance overtakes that of the genetic variance. As the more slowly growing seedlings also capture their site, inter-tree competition is intensified. During the maturing linear growth phase, trees that are genetically superior in the competitive condition have a greater constant growth rate than those that are less competitive. Populational differences strongly emerge, but the error variance is restricted. Therefore, another genotypic culmination can be observed at this phase. During the third period (senescent deceleration phase) growth rate is reduced, but the decreasing rate may be different for different trees. When the rapidly developing phenotypes are slowing down

---

[1] Professor, Department of Forestry, Southern Illinois University, Carbondale, IL, 62901. The author is grateful to Dr. Osborn O. Wells and the Southern Forest Experiment Station for providing the data for this study.

faster than the less rapidly developing phenotypes, heritability starts to fall. Finally, when the carrying capacity is reached, the over-mature forest enters the phase of constancy. Therefore, it is natural for tree breeders to assume that growth rate and acceleration of growth rate is different among different seed sources at different phases of stand development. The first objective of this paper is to test whether or not such a hypothesis is true for the loblolly pine. In economic analysis of tree improvement program, it is also important to know the cumulative growth curve and the annual increment growth curve. When a forest is under sustained yield management, the rotation age is set at the maximum mean annual increment. It can be determined by drawing a tangent from the origin to the growth curve. The rotation age is logically inside the senescent deceleration phase. Therefore, we should consider both growth rate and deceleration of growth rate in a tree improvement program.

In this paper, the Loblolly pine south-wide study is used to illustrate the racial difference in growth rate and in growth rate of growth rate (acceleration or deceleration), as well as the relationship between the first and the second derivatives of the growth curve.

SOUTH-WIDE LOBLOLLY PINE SEED SOURCE STUDY

Complete details of the Loblolly pine experiment are given by Wells and Wakeley (1966). Fifteen seed sources are represented and 15 plantings survived after 25 years in the field.

Measurements of total height were made on each tree at various ages from 1 to 27 years. We used all available trees measured in 3, 5, 10, 15, 20, and 25 years to calculate the mean height growth for each seed source. The average number of trees in each seed source was about 500 for the range-wide study. Means of the 15 seed sources are listed in Table 1. The mean standard deviation and the coefficient of variation are also presented.

ACCELERATION MODEL FOR GROWTH

Most growth models describing size at age data are associated with growth rate (actural increment) or relative growth rate (increment relative to size). For example, when an essential factor for growth is limiting and conserved at a constant value, the actural increment is constant and the growth model is a linear model. Without limiting factors and with a constant relative growth rate, it is an exponential model. With limiting factors acting on the growth rate itself, the model is monomolecular. If the limiting factor is affecting the relative growth rate, the model becomes a logistic model. Finally, the Bertalanfly model related growth rate to both catabolism and anabolism (Savageau, 1979).

Because growing is a dynamic process, one may consider the seemingly abstract question: "What is the relative growth rate of the relative growth rate?"

Table 1.--South-wide regional means of height growth among loblolly pine seed sources.

Seed Source	Regional Mean at Age					
	3	5	10	15	20	25
	- - - - - - - - - - - - - - - - 0.01 ft.- - - - - - - - - - - - - - - -
301	451	1089	2872	4174	5270	6092
303	455	1092	2907	4283	5388	6339
305	479	1137	3021	4440	5582	6410
307	391	948	2566	3907	5078	5907
309	481	1120	2866	4166	5331	6305
311	388	940	2599	3955	5146	5964
315	432	1043	2716	4085	5133	6007
317	381	926	2518	3821	4928	5865
319	457	1057	2790	4113	5243	6069
321	377	903	2545	3898	4976	6028
323	433	1079	2897	4244	5317	6162
325	438	1048	2793	4020	5006	5849
327	412	992	2654	3782	4843	5681
329	393	934	2608	3779	4831	5643
331	346	905	2585	3915	4912	5949
Mean	421	1014	2729	4039	5132	6018
St. Dev.	40	82	159	197	220	222
C.V. %	9.5	8.1	5.8	4.9	4.3	3.7

To illustrate, let us assume that the size of a tree at age t is Y(t); then the growth rate is the difference between two sizes for a given time period, dY/dt. Dividing the increment by its size, the relative growth rate is obtained. For example, in Table 2, the sizes (column 2) at age 1 and age 2 are respectively 100 and 200, hence the growth rate (column 3) is 100, and the relative growth rate (column 4) is unity. Furthermore, let us denote the relative growth rate by Z, the difference between two Z's is dZ, then, dZ/dt is called acceleration in column 5 of Table 2. Dividing acceleration by its relative growth rate in column 6 we have a term called the relative acceleration rate or "the relative growth rate of the relative growth rate (dZ/Z)." If we denote the relative acceleration rate by X, then we have the following relationship:

$$Z = dY/(Ydt)$$

$$\text{and} \quad X = dZ/(Zdt)$$

Thus, the acceleration model concerns the modeling of X.

The simplest model for the relative acceleration rate X is to decompose it into two components: a constant (B0) and an incremental relative acceleration (B1) * Z:

$$X = -(B0 + B1 * Z)$$

where B0 and B1 are regression coefficients. For convenience, the model is written with a minus sign on the right-hand side because decceleration is more common than acceleration. For example, the value of X in the 5th column of Table 2 can be expressed as X = -(.1 + .1Z).

Table 2.--Simulated example to illustrate the relationship among growth rates and acceleration of growth rates.

(1)	(2)	(3)	(4)	(5)	(6)
		Growth Rate		Acceleration Rate	
Age t	Size Y	Actual dY/dt	Relative Z=dY/(Ydt)	Actual dZ/dt	Relative X=dZ/(Zdt)
1	100.00				
		100.00	1.0000		
2	200.00			-0.2000	-.2000
		160.00	0.8000		
3	360.00			-0.1440	-.1800
		236.16	0.6560		
4	596.16			-0.1086	-.1656
		326.32	0.5474		
5	922.48			-0.0847	-.1547
		426.80	0.4627		
6	1349.28			-0.0677	-.1463
		532.96	0.3950		
7	1882.24			-0.0551	-.1395
		639.76	0.3399		
8	2522.00			-0.0455	-.1340
		742.36	0.2944		
9	3264.36			-0.0381	-.1294
		836.50	0.2563		
10	4100.86				

Given the model as above, what would be the model for the size Y(t) and the relative growth rate Z(t) at a given time at T?

To simplify calculation in modelling, let us use * for multiplication and ** for power function and denote the following terms:

```
Y1B = Y1 ** B1
Y2B = Y2 ** B1
E1N = EXP {-B0 * (T - T1)}
E1P = EXP { B0 * (T - T1)}
E21 = EXP {-B0 * (T2 - T1)}
E2T = EXP {-B0 * (T2 - T)}
```

Here, T1 and T2 are the ages at the beginning and at the end of the growth model. Y1 and Y2 are the expected sizes at the beginning and at the end of

the growth model. Y1, Y2, B0, and B1 are four parameters to be estimated in the nonlinear regression model for total growth Y(t):

$$Y(t) = \{Y1B + (Y2B - Y1B) * (1 - E1N)/(1 - E21)\} ** (1/B1)$$

The input data points are observed sizes and ages (Y(t) and T).

After the four parameters Y1, Y2, B0 and B1 are obtained from fitting the total growth model, the relative growth rate at time T can be expressed as:

$$Z(t) = (B0/B1) * (Y2B - Y1B)/\{Y2B * (E1P - 1) + Y1B * (1 - E2T)\}$$

The solutions to Y(t) and Z(t) are due to Schnute (1981).

FITTING THE MODEL

Fitting the acceleration model to data from Table 1 was carried out by PROC NLIN (SAS., 1985). The initial values used for the nonlinear regression fitting were as follows:

Y1 = 420
Y2 = 6020
B0 = 0.06
B1 = 0.8

The initial value for Y1 and Y2 were obtained from the grand mean of height at age 3 and 25 years, while those for B0 and B1 were by experience. With these initial values, the model converged rapidly. It took less than one second of the computer time to complete the NLIN procedure.

The nonlinear regression parameters and their standard error were listed in Table 3. All regression coefficients are significantly different from zero, but not all seed sources have the same coefficients. The coefficients Y1 and Y2 in Table 3 are very close to the regional means at age of 3 and 25 years in Table 1 respectively.

The degree of determination (R-square) for modeling the height growth of the loblolly pine was extremely high. In all 15 cases, the R-square was more than 0.9999. On the other hand the root mean square of fitting error was very low. In no cases would it exceed 0.1 feet.

RESULTS AND DISCUSSION

By inserting the value of Y1, Y2, B0 and B1 into the equation for the relative growth rate, and at the same time, entering the value of T from 4 to 24 in step of 2, we have the projected relative growth rates for the 15 seed sources of loblolly pine in Table 4.

Table 3.--Estimates of regression parameters and standard errors for the acceleration model.

Seed Source	Estimate (standard error) of Coefficient			
	Y1	Y2	B0	B1
305	460 (44)	6402 (44)	.0736 (.0124)	.718 (.122)
303	429 (60)	6317 (60)	.0555 (.0167)	.840 (.166)
309	459 (59)	6293 (58)	.0416 (.0167)	.930 (.171)
323	411 (51)	6146 (50)	.0657 (.0143)	.795 (.140)
301	431 (52)	6083 (52)	.0632 (.0150)	.806 (.150)
319	439 (45)	6065 (45)	.0654 (.0138)	.742 (.137)
321	347 (70)	5999 (71)	.0449 (.0212)	.833 (.206)
315	418 (32)	5993 (71)	.0625 (.0098)	.765 (.097)
311	378 (40)	5970 (40)	.0706 (.0128)	.646 (.123)
331	314 (89)	5909 (88)	.0448 (.0254)	.890 (.244)
307	383 (33)	5913 (33)	.0666 (.0107)	.673 (.103)
317	362 (38)	5854 (38)	.0501 (.0121)	.803 (.118)
325	410 (73)	5823 (72)	.0586 (.0213)	.867 (.213)
327	385 (80)	5668 (78)	.0464 (.0238)	.930 (.240)
329	363 (75)	5630 (74)	.0577 (.0232)	.823 (.228)
Combined	396 (59)	6062 (58)	.0498 (0171)	.859 (.170)

Table 4.--Computed relative growth rates of loblolly pine seed sources.

Seed Source	Age										
	4	6	8	10	12	14	16	18	20	22	24	ave.
305	1.342	.415	.230	.152	.109	.082	.064	.051	.041	.034	.028	.232
303	1.158	.365	.207	.139	.102	.079	.062	.051	.042	.035	.030	.206
309	1.053	.337	.193	.132	.099	.077	.062	.052	.043	.037	.032	.193
323	1.217	.379	.213	.142	.102	.078	.061	.049	.040	.033	.028	.213
301	1.202	.376	.211	.141	.102	.078	.062	.050	.041	.034	.028	.211
319	1.304	.407	.228	.152	.110	.084	.066	.053	.043	.036	.030	.228
321	1.174	.347	.214	.146	.108	.084	.068	.056	.047	.040	.034	.213
315	1.267	.396	.223	.149	.108	.083	.065	.053	.043	.036	.030	.223
311	1.494	.463	.258	.171	.123	.093	.073	.058	.047	.039	.032	.259
331	1.099	.350	.200	.137	.101	.079	.064	.053	.044	.037	.032	.200
307	1.437	.447	.250	.167	.121	.092	.072	.058	.047	.039	.032	.251
317	1.214	.385	.219	.149	.110	.085	.068	.056	.046	.039	.033	.219
325	1.120	.352	.199	.133	.097	.075	.059	.048	.040	.033	.028	.198
327	1.051	.334	.191	.130	.096	.075	.060	.050	.042	.035	.030	.190
329	1.180	.371	.210	.141	.103	.079	.063	.051	.042	.035	.030	.210
All	1.135	.360	.205	.139	.103	.079	.064	.052	.044	.037	.031	.204

The seed sources in Table 3 and in Table 4 were ordered according to their heights at the age of 25 years. There seems to be no correlation between heights at any given age and the average relative growth rate. For example, seed source No. 311 which had the greatest average rate of .259 was ranked below average in height. On the other hand, seed source No. 309 which was ranked in the top 20th percentile in height growth would be in the lower 20th percentile of average growth rate. The reason for this lack of consistency may be due to the fact that seed source No. 309 was the tallest one at age 3. Therefore, even with the below average growth rate it continued to have better than average performance. On the other hand, seed source No. 311 which were 10% below average at the beginning would take a long time to overcome the initial slow start even with a superior rate of growth increment.

Another interesting contrast was found between seed sources No. 305 and 309. The initial heights at age 3 for these two seed sources were the same and the final heights at age 25 differed by less than 2%. However, the relative growth rate curves for these two seed sources were quite different. Before age 17, seed source No. 305 had a faster rate than seed source No. 309, but the growth trend reversed itself after that point in time. It can be seen in Table 3 that seed sources No. 305 and No. 309 have different constant acceleration rates (B0) and different incremental relative acceleration rates (B1). The constant acceleration rate is smaller, but the incremental acceleration rate is greater for seed source No. 309 than seed source No. 305.

Differences in the relative growth rates among seed sources through the age periods probably reflect different competition abilities. Seed sources No. 311 and No. 307 were good competitors and seed source 325, 327 and 329 were poor competitors throughout the study period of 4-24 years. Seed source No. 305 and No. 319 were good only before age 15 and seed source 321 had the best growth rate in the later years.

Differences in the acceleration parameter B0 and B1 indicate differences in the shape of growth curve among 15 seed sources of loblolly pine. Past studies of growth curves were seldom able to detect the shape differences. For example, in fitting the Weibull function to five half-sib progeny tests, site had no significant effect on the shape parameter. Significance was found in the check-vs.-selection, but not among selections (Spirek et al., 1981). Failure to find significant differences in the shape parameter of the Richard's nonlinear function among populations of Douglas-fir had been reported (Namkoong et al., 1972). The success of detecting shape difference in this study possibly is due to the solid data base and the versatile growth model. First, the regional means with a large sample size over a broad planting area were used. This effectively removed the sensitivity to outlying trees. Secondly the Schnute's model is versatile and with statistically stable parameters (Schnute, 1981).

The average relative growth rate was found to be positively correlated with the acceleration rate ($r = .74$), but negatively correlated with the incremental relative acceleration rate ($r = -.98$). The coefficient of acceleration rate (B0) and of incremental rate (B1) were also negatively

correlated (r = -.85). Thus, selection for fast growth rate would likely result in simultaneous selection for high decreasing rate of the relative growth rate. The "constant relative rate of relative growth rate" and the "incremental relative rate of relative growth rate" are in check-and-balance.

LITERATURE CITED

Franklin, E.C. 1979. Model relating levels of genetic variance to stand development of four North American conifers. Silvae Genet. 28: 207-212.

Husch, B., C.I. Miller, and T.W. Beers. 1972. Forest Mensuration, 2nd ed. Ronald Press. New York. 410 pp.

Namkoong, G., R.A. Usanis and R.R. Silen. 1972. Age-related variation in genetic control of height growth in Douglas-fir. Theo. Appl. Genet. 42 :151-159.

SAS. 1985. SAS User's Guide: Statistics Version 5 ed. SAS Inst. Inc. Cary, NC. 957 pp.

Savageau, M.A. 1979. Growth equations: A general equation and a survey of special cases. Math. Biosciences. 48: 267-278.

Schnute, J. 1981. A versatile growth model with statistically stable parameters. Can. J. Fish. Aquat. Sci. 38: 1128-1140.

Spirek, F.J., L.G. Arvanitis, D.L. Rockwood and R.C. Littel. 1981. Modeling diameters and heights of improved slash pine (pinus elliottii Engelm. var. elliottii) using Weibull distributions. South. For. Tree. Improv. Conf. 16: 47-54.

Switzer, G.L. and M.G. Shelton. 1981. Stand productivity: Some considerations in evaluating genetic improvement. South. For. Tree Improv. Conf. 16: 18-32.

Wells, O.O. and P.C. Wakeley. 1966. Geographic variation in survival, growth and fusiform-rust infection of planted loblolly pine. For. Sci. Monogr. 11:40.

GEOGRAPHIC GENETIC VARIATION IN SOUTHERN RED OAK

A. M. Mukewar[1/] and S. B. Land, Jr.[2/]

Abstract.--A five-year-old southern red oak provenance-progeny test planted in central Mississippi exhibits clinal patterns of increasing tree height, diameter, and survival as origin of the acorns moves southward and/or closer to the sea coast. Sources from southeastern Texas and eastern Georgia have grown fastest at the Mississippi site. Narrow-sense heritability estimates for open pollinated families within sources ranged from 0.30 to 0.43 for third-year and fifth-year diameters and heights. Survival did not differ among provenances during the first five years of the study.

Additional keywords: Quercus falcata var. falcata, provenance test, progeny test, heritability.

The typical variety of southern red oak (Quercus falcata Michx. var falcata) is one of the most ubiquitous of the upland southern oaks. Its range extends from New York to northern Florida and westward to eastern Texas and Oklahoma (Fowells 1965). Although it is usually not as valuable per tree as its related variety, cherrybark oak (Q. falcata var. pagodaefolia), the lumber of equivalent grade from the two varieties is equal in value and is mixed at the sawmill. The combination of (1) high value of oak and (2) frequent occurrence of the typical southern red oak variety makes it an economically important component of the southern forests.

Provenance and progeny studies have been conducted for northern red oak (Quercus rubra L.) (Kriebel 1965, Taft 1965, Webb 1970) and cherrybark oak (Randall 1973). However, no information is available about geographic genetic variation in typical southern red oak or about genetic variation among trees within provenances of the species. The purposes of the study reported here are to (1) describe patterns of geographic genetic variation in the southern population of the species (2) identify the fastest growing sources for central Mississippi, (3) compare the relative magnitudes of genetic variation within provenances as versus genetic variation among provenances, and (4) provide estimates of heritabilities for height and diameter growth of trees within provenances.

1/Visiting Scientist from India under USAID program, Department of Forestry, Mississippi State University, Mississippi State, MS 39762 (Associate Professor of Forestry, P.K.V. Akola, MS, India 444104).

2/Professor, Department of Forestry, Mississippi State University, Mississippi State, MS 39762. Contribution No. 6699 of the Mississippi Agricultural and Forestry Experiment Station.

MATERIALS AND METHODS

Open-pollinated acorns from 112 southern red oak trees representing 43 stands in 23 provenances (Figure 1) were collected in 1979 and kept sepa-

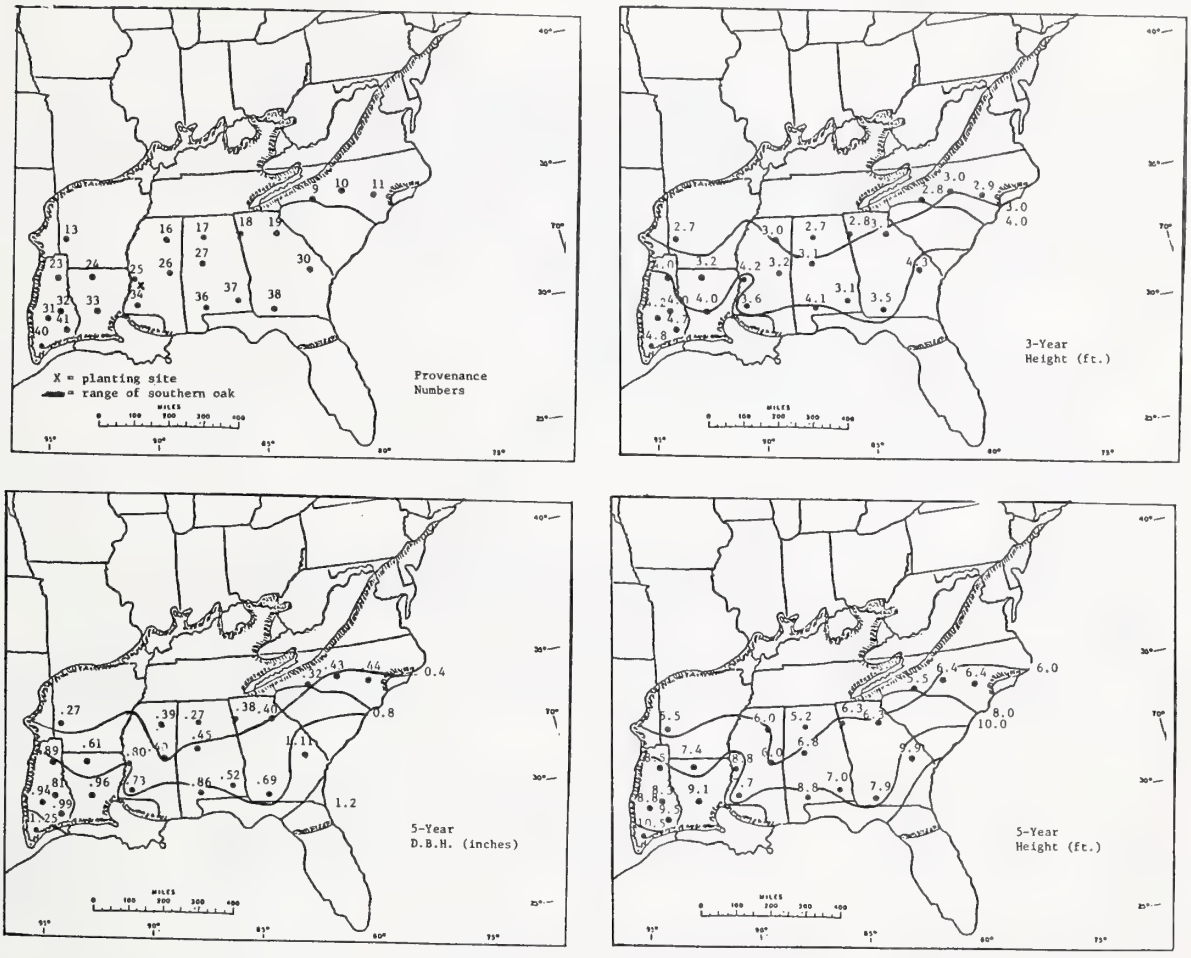


Figure 1. Maps of the natural range of southern red oak with locations of provenances and patterns of geographic genetic differences for third-year height, fifth-year DBH, and fifth-year height.

rately identified by mother tree. Provenances ranged in latitude from 29.7°N (Harris County, Texas) to 35.4°N (Montgomery County, North Carolina), and in longitude from 78.3°W (Sampson County, North Carolina) to 95.4°W (Harris and Trinity Counties, Texas).

Seedlings were raised during 1980-81 at the USDA Forest Service nursery in Starkville, Mississippi. In February 1982 the 2-0 seedlings were outplanted at the MAFES (Mississippi Agricultural and Forestry Experiment Station) Truck Crops Branch Experiment Station near Crystal Springs,

Mississippi. Spacing among trees is 10 x 10 feet and the planting is surrounded by two border rows. The experimental design is a randomized complete block with five replications and three-tree family row plots. The row plots of families from the same provenance were planted adjacent to each other within a replication, so that analysis of variance will treat the study as a split plot design. Seed sources are the whole units and families within sources are the subunits. The design is called a compact family block design (Snyder 1966).

Observations of survival were taken after the first and second growing seasons in the field. Groundline diameter and height of each tree were recorded after the third growing season. Survival, d.b.h., and height were measured after five growing seasons in the field. Data were analyzed by SAS computer software (SAS Institute 1985). All effects were considered random in the analyses of variance (Table 1), and a Satterthwaite-F (pseudo-F) test (Hicks 1973) was used for the "provenance" source of variation. Patterns of

Table 1. Format of analysis of variance used for a southern red oak provenance-progeny test.

Sources of Variation	D.F.	E.M.S.[a/]
Replications (=R)	4	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}+f\sigma^2_{RS(P)}+fS\sigma^2_{RP}+fSP\sigma^2_R$
Provenances (=P)	22	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}+f\sigma^2_{RS(P)}+r\sigma^2_{F(SP)}+rf\sigma^2_{S(P)}+fs\sigma^2_{RP}+fsr\sigma^2_P$
RxP	88	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}+f\sigma^2_{RS(P)}+fs\sigma^2_{RP}$
Stand w/i Prov (=S/P)	20	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}+f\sigma^2_{RS(P)}+r\sigma^2_{F(SP)}+rf\sigma^2_{S(P)}$
Families w/i Stand w/i Prov (=F/S/P)	69	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}+r\sigma^2_{F(SP)}$
RxS/P	80	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}+f\sigma^2_{RS(P)}$
RxF/S/P	276	$(1/h)\sigma^2_w+\sigma^2_{RF(SP)}$
Among Trees w/i Plots	1120	σ^2_w
Total	1679	

[a/] h = harmonic mean number of trees per plot.

geographic genetic variation were illustrated with isogenic lines drawn on a range map of the species in the southern United States, and Students t-test was used to determine significant differences of provenance means from the overall study mean. Narrow-sense heritability estimates on an individual-tree basis were determined from family variation within stands within provenances by the equation

$$h^2_N = \frac{4\sigma^2_{F(SP)}}{\sigma^2_w + \sigma^2_{RF(SP)} + \sigma^2_{F(SP)}},$$

where the variance components were determined by equating actual mean squares with the expected mean squares in Table 1.

RESULTS AND DISCUSSION

Overall survival for the study was 93 percent after five growing seasons in the field (Table 2), so that no significant differences were

Table 2. Southern red oak study means, coefficients of variation, and ranges in provenance means for survival, stem diameter, and height.

	Percent Survival			Third Year		Fifth Year	
Statistic	1st Yr.	2nd Yr.	5th Yr.	Groundline Dia. (in.)	Ht. (ft.)	DBH (in.)	Ht. (ft.)
Study Mean	95	94	93	0.95	3.51	0.63	7.42
C.V. for Plot Means	13.50	15.96	16.38	23.17	18.08	41.66	18.33
Range in Prov. Means	89-100	87-97	80-87	0.65-1.41	2.69-4.84	0.27-1.25	5.21-10.56

detected among sources, stands, or families for this trait. Mean height for the study doubled from 3.5 feet at age three (three growing seasons in the field) to 7.4 feet at age five, and the error variance increased by about the same amount as indicated by essentially no change in the coefficient of variation. By age five the average tree had a DBH of nearly two-thirds of an inch, but values varied widely as illustrated by the large coefficient of variation and wide range in source means.

Genetic variation among the 112 families in the study was significant for both diameter and height at both age three and age five (Table 3). Provenance effects accounted for approximately 70 percent of this variation, and family differences within stands within provenances made up another 20 percent. However, variation among local stands within provenances was only significant for root collar diameter at age three, and it accounted for only

Table 3. Variance component estimates and narrow-sense heritabilities for third-year and fifth-year diameters and heights in the southern red oak study.

Trait	h^2_N	Variance Components and Results of F-tests[a/] (1) Provenance Estimate (% Total)	(2) Stand w/i Prov. Estimate (% Total)	(3) Family w/i Stand Estimate (% Total)	Total Family Variance (1+2+3)
Root Collar Age 3	0.30	0.0301** (70)	0.0043** (10)	0.0088** (20)	0.0432
Height Age 3	0.40	0.3350** (67)	0.0612 ns (12)	0.1043** (21)	0.5005
D.B.H. Age 5	0.40	0.0700** (73)	0.0085 ns (9)	0.0170** (18)	0.0955
Height Age 5	0.43	1.9635** (72)	0.2462 ns (9)	0.5161** (19)	2.7258

a/** = significant at 99% level of probability;
* = significant at 95% level of probability;
ns = nonsignificant.

ten percent of the family variability. A similar lack of genetic differences among local stands has been reported by Land (1981) for sycamore (Platanus occidentalis L.). Migration probably predominates over local natural selection forces (but not regional natural selection) in forming patterns of genetic variation in these species' natural populations.

The significant genetic variation among families within stands within provenances indicates that genetic gains in growth can be accomplished from selection of individual trees within stands to serve as parents for a seed orchard. The amount of gain is dependent upon the size of the narrow-sense heritability. In a provenance-progeny test the narrow-sense heritability must be based on the additive genetic variance among trees from the same breeding population (families within stands within provenances), since crosses among different provenances may not give a purely additive response. Narrow-sense heritability estimates in the present study are near 0.40 (Table 3), which are quite high. These estimates may be inflated from genotype-by-site interactions, since the study was grown on only one site, or by the incorrect assumption that all individuals of a family are half sibs. Squillace (1974) has shown for open-pollinated families that a better approximation of additive genetic variation is three times the family variance component, rather than four times the component. With this

adjustment and some reduction for genotype-by-site interactions, the heritabilities might be halved to give values near 0.20. These values are close to those found for other species and can still provide genetic gains from mass selection.

The significant variation among provenances indicates that additional genetic gains at the central Mississippi site can be obtained by using acorns collected from the best geographic origins. Ranked provenance means show that progenies obtained from southeast Texas (#40 from Harris County and #41 from Jasper County) and eastern Georgia (#30 from Bulloch and Candler Counties) grow significantly faster than the study average at the Crystal Springs site (Table 4). Other provenances that fall in the superior

Table 4. Ranked provenance means for third-year and fifth-year diameters and tree heights in the southern red oak provenance-progeny test. Only those provenances that are significantly greater (5% probability level) than the study mean are given.

Third Year						Fifth Year					
Root Collar Diam. (in.)			Height (ft.)			DBH (in.)			Height (ft.)		
Source	Rank	Mean	Source	Rank	Mean[a]	Source	Rank	Mean	Source	Rank	Mean
40	1	1.41	40	1	4.84	40	1	1.25	40	1	10.50
			41	2	4.68	30	2	1.11	30	2	9.94
			30	3	4.32	41	3	0.99	41	3	9.50
			25	4	4.24	33	4	0.96			
			31	5	4.20						
Study Mean = 0.95			Study Mean = 3.51			Study Mean = 0.63			Study Mean = 7.42		
CD = 0.23[b]			CD = 0.66			CD = 0.27			CD = 1.41		

[a] Lines connect means that are not significantly different at the 5% probability level according to the Student-Neuman-Keuls test.

[b] CD = critical difference = SEM x "t" (having error df at 5%), where SEM = $\sqrt{2 \times (\text{error SS/DF})/(\text{No. of Reps})}$

group for at least one of the growth traits include #33 from Rapides Parish in Louisiana, #31 from Trinity and Polk Counties in Texas, and #25 from Warren County in Mississippi. The sources #40, #41, and #30 all come from within 50 to 75 miles of the coast and probably represent adaptation to a

milder climate with a longer growing season than found for the other sources. However, sources #31 and #33 are only 100 miles from the Texas-Louisiana coast and are south of the planting site, so that they are also probably adapted to a long growing season. Source #25 is slightly north of the planting site, but the trees come from near the Mississippi River. A possibility for its superior performance is discussed below.

A better picture of patterns and possible causes of geographic genetic variation in southern red oak is obtained by mapping the provenance locations and means and then drawing isogenic lines (Figure 1). The lines indicate that when trees are planted in central Mississippi, there is a clinal increase in growth as geographic origin of the acorns moves south in latitude or towards the coast at the same latitude. Trees from milder oceanographic climates grow more per year than trees from colder continental climates when planted at that site. The one abnormality in this trend is an apparent bulge in isogenic lines up the Mississippi River. One would have anticipated that the bulge would be in the opposite direction due to migration (seed movement) down the river. That type of response and hypothesis was given by Wells et al. (1979) for sweetgum (Liquidambar styraciflua L.) in Mississippi. Perhaps the reason for the faster growth of "Mississippi River neighborhood sources" than other sources at the same latitude is hybridization with cherrybark oak. This possibility has not yet been investigated.

CONCLUSIONS

The geographic area of acorn collection had no effect on survival of southern red oak for the first five years after planting in central Mississippi. Provenance differences and family-within-stand-within-provenance differences for stem diameter and height growth were highly significant, however, and indicated a great amount of genetic diversity within the species' natural population. Provenances from southeast Texas and eastern Georgia proved to be the fastest growing sources when planted in central Mississippi. It is believed that these sources have evolved in milder climates and have a longer growing season than other sources in the study. Hybridization with cherrybark oak is proposed as a possible reason for the good performance of sources from near the Mississippi River.

LITERATURE CITED

Fowells, H. A. 1965. Silvics of Forest Trees of the United States. USDA Handb. 271. 762 pp.

Hicks, C. R. 1973. Fundamental Concepts in the Design of Experiments. Holt, Rinehart, and Winston Co., New York. 349 pp.

Kriebel, H. B. 1965. Parental and provenance effects on growth of red oak seedlings. Fourth Central States For. Tree Improv. Conf. Proc., p. 19-28.

Land, S. B., Jr. 1981. Genetic variation, heritabilities, and selection strategies for early growth of sycamore in the Gulf South. Proc. Sixteenth Southern For. Tree Improv. Conf. Proc., VPI&SU, Blacksburg, VA. p. 123-135.

Randall, W. K. 1973. Early results from a cherrybark oak improvement project. Proc. Twelfth South. For. Tree Improv. Conf. pp. 181-184.

SAS Institute. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute, Inc., Cary, NC. 956 pp.

Snyder, E. B. 1966. Lattice and compact family block designs in forest genetics. In: Joint Proc. 2nd Genetics Workshop of Soc. Am. Foresters and 7th Lake States Tree Improv. Conf., p. 12-17. Res. Paper NC-6. USDA Forest Service, North Central For. Exp. Sta., St. Paul, MN.

Squillace, A. E. 1974. Average genetic correlations among offspring from open-pollinated forest trees. Silvae Genetica 23(5):149-156.

Taft, K. A., Jr. 1965. Improvement of oaks. Proc. Eighth Southern Conference on Forest Tree Improvement. p. 52-55.

Webb, C. D. 1970. Genetics and tree improvement in the oak-yellow poplar type. USDA For. Service Res. Paper NE-144: "The silviculture of oaks and associated species." p. 57-66.

Wells, O. O., G. L. Switzer, and W. L. Nance. 1979. Genetic variation in Mississippi sweetgum. Proc. Fifteenth Southern Forest Tree Improvement Conference, Mississippi State University, Mississippi State, MS. p. 22-32.

TESTS OF REALIZED GENETIC GAIN FROM A COASTAL VIRGINIA LOBLOLLY PINE FIRST GENERATION SEED ORCHARD

G.A. Lowerts[1/]

Abstract:--Percent volume gain observed in the performance of improved loblolly pine (Pinus taeda L.) mix seedlots at five locations ranged from -3 to +6 compared to the volume growth of commercial check seedlots. The improved mix seedlots were obtained from several seed collection years in a first generation seed orchard containing clones that originated from the Coastal Plain of southeastern Virginia and northeastern North Carolina. The data were obtained from eight- and four-year-old block plot tests in which the objective was to determine realized genetic gain from seed orchards.

INTRODUCTION

Realized genetic gain from seed orchards can be determined only by using appropriately designed field tests. Meaningful estimates of gain on a per acre basis can only be calculated from block plot tests of improved and unimproved seedlings (Zobel and Talbert, 1984). After thirty years of active tree improvement involving many industrial corporations and government agencies in the Southeast, there is surprisingly few published reports of block plot estimates of realized genetic gain. A volume gain of 20 percent in a rogued first generation loblolly seed orchard mix (origin: Coastal Georgia and South Carolina) was reported by Lowerts (1986) in a six-year-old test of realized gain. This paper will describe the gain in volume observed in eight- and four-year-old block plot test of realized genetic gain from a first generation loblolly pine seed orchard containing clones selected from natural stands in the Coastal Plain of Virginia and northeastern North Carolina.

EXPERIMENTAL METHODS

1979 TEST OF REALIZED GENETIC GAIN

In March, 1979, three locations of a loblolly pine test of realized genetic gain were established on a moderately well drained Goldsboro soil in Prince George and Isle of Wight Counties, Virginia (location 1 and 2, respectively) and on a moderately well drained Duplin soil in Hertford County, North Carolina (location 3). Eight replications of six or seven seedlots were planted (bareroot seedlings) at each location in 25-tree-block

---

[1/]Forest Geneticist and Seed Orchard Manager, Woodlands Research, Union Camp Corporation, Rincon, Georgia 31326, USA.

(5 x 5) plots at a spacing of 10 x 6 feet. The seedlots were:

1. An unimproved commercial check seedlot collected in 1975 by the North Carolina Forest Service (CCNCF), origin unknown.

2. An unimproved commercial check seedlot collected in 1967 by the Virginia Division of Forestry (CCVDF), origin known.

3. An unimproved commercial check seedlot collected by Union Camp Corporation (CCUCC) on Company lands in southeastern Virginia.

4. Three unrogued first generation seed orchard mix seedlots collected from the Murfreesboro Seed Orchard, Hertford County, North Carolina in 1973, 1975, and 1976 (SOM73, SOM75, SOM76), respectively. The clones in the orchard originated from wild stand selections in Southeast Virginia and Northeastern North Carolina.

5. A seed orchard mix seedlot collected in 1976 from a rogued block of the Murfreesboro Seed Orchard (SOMR76). Spacing and seed production were the primary considerations for rogueing.

Because of a shortage of plantable seedlings, the SOM75 seedlot was not planted in location 1 and the CCVDF seedlot was not planted in location 2.

Site preparation of all locations consisted of KG and rake. Each seedling at each location of the 1979 test received six grams of Furadan, 10g and four ounces of 8-40-0 fertilizer. Seven years after planting (1986), herbicide (Velpar, L, 0.75 lb./acre) was applied to each location. Each location was mowed in 1981 and 1985.

1983 TEST OF REALIZED GENETIC GAIN

In February, 1983, two additional locations of a test of realized genetic gain from the Murfreesboro Seed Orchard were also established on a somewhat poorly drained Augusta soil in Isle of Wight County (location 1) and on a well drained Wagram soil in Southampton County (location 2), Virginia. Each location contained six replications of two different seedlots planted (bareroot seedlings) in 49-tree-block (7 x 7) plots at a spacing of 9 x 9 feet. The two seedlots were: 1. an unimproved commercial check mix seedlot, and 2. a first generation improved mix seedlot, Murfreesboro 1979 Group A. The commercial check mix seedlot was composed of seed obtained from the CCNCF, CCUCC, and CCVDF seedlots. The Murfreesboro Group A seedlot is a mix of the seed from the best performing clones in the seed orchard based on the 1978 North Carolina State University - Industry Tree Improvement Cooperative performance levels. The Murfreesboro Group A seedlot is not representative of an orchard rogued for genetic quality since the Murfreesboro orchard still contained many inferior clones in 1979. The seed from these poor performing clones was collected as Group B.

Site preparation of all locations consisted of KG and rake. Each seedling of each location received 10 grams of Furadan, 10G at the time of planting. Fertilizer was not applied to either location. Two years after planting, herbicide (Velpar, L, 0.75 lb./acre) was applied to each location. Location 1 was mowed in 1984 and 1986 and location 2 was mowed in 1986.

In the winter of 1987, total tree height, diameter (at breast height), and fusiform rust incidence were measured on the nine (3 x 3), and twenty-five (5 x 5) interior trees of the 1979 and 1983 tests, respectively. A meaningful estimate of diameter was unavailable in location 1 of the 1983 test of gains because of small tree size.

Statistical Procedure

Seedlot mean total height, diameter (DBH), percent fusiform rust infection, and percent survival were determined for each replication at each location using individual tree measurements. The incidence of fusiform rust was very low among all seedlots at all locations of both tests and was not considered for further analysis. Seedlots were analyzed on an individual basis or as the mean of improved and commercial check seedlots. The percent data was transformed by the arcsine procedure prior to the analysis of variance. Individual tree volume was calculated using the formula: Volume = 0.03371 + 0.001961128*(Dia.*Dia.*Height) (Goebel and Warner, 1962). The total seedlot volume for each replication and each location was determined by summing the individual tree volumes. Total volume of each seedlot was adjusted for differences in survival. The combined analysis of variance for the 1979 and 1983 tests used location, seedlot, location*seedlot, and replication within location as the source of variation. For both tests, the results of the combined analysis revealed a highly significant (p=0.01) location effect for all variables except survival. In the 1979 test, height was the only variable that displayed a significant (p=0.01) difference among seedlots in the combined analysis. The location*seedlot interaction of any variable measured was not significantly different for either test. Mean height, and diameter, individual tree volume, and percent survival were then analyzed by location for each test using replication and seedlot as the source of variation.

RESULTS

1979 Test of Realized Genetic Gain

Seedlot mean height and individual tree volume were significantly different at location 1 (Table 1), all other variables at each location were not significantly different among seedlots. At each location, the improved seedlots displayed a slightly greater height than the commercial check seedlots (Table 2). The mean diameter of the improved and commercial check seedlots at each location was very similar. Percent survival was very uniform among the three locations.

Percent mean height gain of the improved seedlots compared to the average performance of the commercial check was +3, +2, and +1 at location 1, 2, and 3, respectively (Table 3). Percent volume gain of the average of the improved seedlots at each location versus the average of the commercial check seedlots was +6, -1, and +1 at locations 1, 2, and 3, respectively. The performance of the improved seedlots varied with the check seedlot used as a comparison. Volume gains of SOMR76 ranged from +2 to +12 percent when compared to CCUCC, and from -10 to +6 percent when compared to CCNCF (Table 4).

Table 1. Analysis of variance for height, diameter, individual tree volume, and percent survival for each location of the 1979 and 1983 tests of realized genetic gain.

Source of Variation	D.F.	Mean Square for Height	Dia.	Ind. Tree Volume	Percent Survival
		1979 TEST OF GAIN, LOCATION I			
Seedlots	5	5.1088^{**}	0.1057^{ns}	0.0289^{*}	0.0060^{ns}
Reps.	7	4.8599^{**}	0.1433^{*}	0.0423^{**}	0.0225^{*}
Error	35	1.2214	0.0569	0.0104	0.0092
		1979 TEST OF GAIN, LOCATION II			
Seedlots	5	1.2569^{ns}	0.0190^{ns}	0.0029^{ns}	0.0035^{ns}
Reps.	7	3.1178^{*}	0.2112^{*}	0.0314^{**}	0.0021^{ns}
Error	35	1.3592	0.0791	0.0099	0.0019
		1979 TEST OF GAIN, LOCATION III			
Seedlots	6	1.7982^{ns}	0.0924^{ns}	0.0264^{ns}	0.0009^{ns}
Reps.	7	4.0849^{**}	0.1635^{*}	0.0436^{**}	0.0183^{*}
Error	42	1.1065	0.0618	0.0130	0.0079
		1983 TEST OF GAIN, LOCATION I			
Seedlots	1	0.0428^{ns}	n.a.	n.a.	0.0012^{ns}
Reps.	5	0.1175^{ns}	n.a	n.a	0.0005^{ns}
Error	5	0.1714	n.a	n.a.	0.0002
		1983 TEST OF GAIN, LOCATION II			
Seedlots	1	0.1520^{ns}	0.0091^{ns}	0.0001^{ns}	0.0012^{ns}
Reps.	5	2.1686^{ns}	0.1876^{*}	0.0014^{*}	0.0018^{ns}
Error	5	0.6875	0.0389	0.0002	0.0050

where ** = significant at the one percent level of probability
* = significant at the five percent level of probability
ns = not significant

Table 2. Improved and commercial check mean height, diameter, and percent survival at each location of the 1979 and 1983 test of realized genetic gain, age 8 and 4, respectively.

Seedlot	Height (ft.)	Diameter (in.)	Percent Survival
		1979 TEST OF GAINS, LOCATION I	
CHECK[1/]	22.4	4.0	97
IMPROVED	23.2	4.0	94
		1979 TEST OF GAINS, LOCATION II	
CHECK	18.9	3.6	99
IMPROVED	19.2	3.6	97
		1979 TEST OF GAINS, LOCATION III	
CHECK	22.7	4.1	95
IMPROVED	23.0	4.1	96
		1983 TEST OF GAINS, LOCATION 1	
CHECK[2/]	6.8	N.A.	99
IMPROVED	6.7	N.A.	97
		1983 TEST OF GAINS, LOCATION 2	
CHECK	10.4	1.7	96
IMPROVED	10.2	1.6	94

1/ Improved seedlot in the 1979 test of gains is the average performance SOM73, SOM75, SOM76, AND SOMR76 and the check seedlot is the average performance of CCUCC, CCNCF, and CCVDF.

2/ Improved seedlot in the 1983 test of gains is the Murfreesboro 1979 Group A mix and the check seedlot is a composite of seed from the CCNCF, CCUCC, and CCVDF seedlots.

N.A. = Not Available

Table 3. Percent volume and height gain of the average total volume and height of the improved seedlots compared to the average total volume and height of the commercial check seedlots at each location of the 1979 (age 8) and 1983 (age 4) tests of realized genetic gain.

Age	Test	Location	% Volume Gain	% Height Gain
8	1979	I	+6	+3
8	1979	II	-1	+2
8	1979	III	+1	+1
4	1983	I	N.A.[1/]	-1
4	1983	II	-3	-2

N.A. = Not Available

Table 4. Percent volume gain from SOMR76 compared to the CCUCC, and CCNCF commercial check seedlots at each location of the 1979 test of gains.

Location	% Volume Gain Vs. CCUCC	% Volume Gain Vs. CCNCF
I	+11	+ 6
II	+ 2	- 3
III	+12	-10

where CCUCC = Union Camp Commercial Check
CCNCF = North Carolina Division of Forestry Check
SOMR76 = Seed Orchard Mix Rogued 1976

1983 Test of Realized Genetic Gain

The improved and commercial check seedlot mean height, diameter (location 2), individual tree volume (location 2), and percent survival were not significantly different at either location (Table 1). The performance of the commercial check exceeded that of the improved seedlot for all variables measured at each location (Table 2). Survival at each location was excellent. Percent mean height gain of the improved over that of the commercial check seedlot was -1 and -2 percent at location 1 and 2, respectively (Table 3). A 3 percent volume loss in the improved seedlot occurred at location 2. The percent volume gains observed in this study should be interpreted with caution since gain estimates at age four and eight years could be influenced to a large extent by the presence of a few big trees (Zobel and Talbert, 1984).

DISCUSSION

Talbert (1982), using row plot data and growth and yield modeling estimated 15- and 25-year-old volume gains from a rogued and unrogued first generation seed orchard to be 20.0 and 12.5, and 10.0 and 6.5 percent, respectively. Realized plantation volume gain from the first generation of tree improvement was estimated by Weir (1973) to be between 10 and 20 percent. These and other theoretical estimates of volume gain have been supported by the 20 percent gain in volume observed in a rogued first generation seed orchard mix seedlot (origin of clones: Coastal Georgia and South Carolina) in a six-year-old block plot test of realized genetic gain (Lowerts, 1986). The volume gains observed in the 1979 and 1983 tests of realized genetic gain are low (-3 to +6 %) when compared to the theoretical gain estimates and previously reported realized volume gains for loblolly pine. The individual improved seedlots in the 1979 test contained seed from many clones which have been rogued from the orchard and approximately 25 percent of the total seed volume in the seedlots could have been supplied by a very poor performing but prolific seed producing clone (2-40) (Stone, 1987). Also, 57 percent of the clones represented in the 1979 Murfreesboro Group A seedlot (1983 test) have been rogued from the orchard. Today, only the 12 best performing of the original 48 clones remain in the Murfreesboro seed orchard and the genetic quality of the seed greatly exceeds that of the seed in the improved seedlots of the 1979 and 1983 tests of realized genetic gain. The presence of seed from the poor performing clones in the improved seedlots in both the 1979 and 1983 tests of gain probably contributed to the relatively low level of observed volume gain.

The results of the 1979 and 1983 tests of realized genetic gain have not clearly established the level of genetic gain achieved by the first generation of tree improvement in the Coastal Virginia area. Also, the results clearly suggest the level of genetic gain observed is dependent on the choice of the commercial check seedlot. The SOMR76 seedlot performed well when compared to the CCUCC seedlot but the volume performance of SOMR76 was not as good when compared to the commercial check seedlot from the North Carolina Forest Service (CCNCF). The CCNCF seedlot performed very well at each location of the 1979 test and probably influenced the growth of the

commercial check mix seedlot in the 1983 test. Unfortunately, the origin of CCNCF in North Carolina cannot be established (Zoerb, 1985). If CCNCF were collected in the southeast coastal area of North Carolina, it would probably possess excellent growth characteristics as displayed in this study since the Onslow County, North Carolina source of loblolly pine was identified by Wells (1983) as a source with good growth potential. The long term adaptability of CCNCF to the environment of Virginia has not been determined. However, through age eight, the CCNCF is performing better than virtually all other seedlots and has displayed excellent survival at each test location suggesting that CCNCF may be adaptable to the environment of Coastal Virginia.

Although positive volume and height gains in the first generation improved seedlots did occur at some locations of the 1979 test, the results of this study suggest the absolute level of genetic gain achieved by the first generation of tree improvement in the coastal region of Southeast Virginia and Northeast North Carolina may not approach the level of genetic gain observed or predicted in other breeding regions. Union Camp Corporation's lands in Virginia and North Carolina are located near the northern fringe of the natural range of loblolly pine. Although experimental evidence is lacking, the opportunity to select outstanding trees for growth characteristics in this region could be reduced because the variation in growth near the northern fringe of the species may be less than that of the southern range. The shorter growing season and harsher winter of Virginia may restrict the expression of growth characteristics, whereas the mild, moist environment of the Atlantic Coast Region of South Carolina and Georgia, where many outstanding selections have occurred, may allow for considerable variation in growth characteristics. The results of this study also suggest that various sources of loblolly pine from North Carolina may perform well in the Coastal Virginia area. A future test of realized gain containing a seed orchard mix of the 12 best remaining clones in the Murfreesboro orchard will provide an estimate of the level of genetic gain achieved when all poor performing clones are rogued.

ACKNOWLEDGMENTS

I thank Marvin Zoerb, Jr. for the foresight to install the realized genetic gain tests, and Lloyd Bass for the excellent quality of his work in establishing, maintaining, and measuring the tests.

LITERATURE CITED

Goebel, T.J. and M.S. Warner. 1962. Volume tables for small diameter loblolly, shortleaf, and Virginia pine in the upper South Carolina Piedmont. Clemson For. Res. Series #7.

Lowerts, G.A.. 1986. Realized genetic gain from loblolly and slash pine first generation seed orchards. IN: Proc. IUFRO Conf. on Breeding Theory, Progeny Testing, and Seed Orchards, Oct. 13-17, Williamsburg, Virginia. pp 142-149.

Stone, J.M.. 1987. Personal communication. Nursery Manager, Union Camp Corporation, Hardwood Nursery, P.O. Box 129, Capron, Virginia 23829.

Talbert, J.T.. 1982. One generation of loblolly pine tree improvement: results and challenges. IN: Proc. 18th Can. For. Tree Improv. Assoc. Meet., Duncan, B.C.. pp 106-120.

Wells, O.O.. 1983. Southwide pine seed source study - loblolly pine at 25 years. South J. Appl. For. 7: 63-71.

Weir, R.J.. 1973. Realizing genetic gains through second generation seed orchards. IN: Proc. 12th South. For. Tree Impr. Conf., June 12-13, Baton Rouge, Louisiana. pp 14-23.

Zobel, B.J. and J.T. Talbert. 1984. Applied Forest Tree Improvement. John Wiley & Sons, New York. 505p.

Zoerb, M.H., Jr.. 1985. Personal communication -- Tree Improvement Project Leader, Woodlands Research Department, Union Camp Corporation, Rincon, Georgia. 31326.

APPENDIX

ADDENDUM

At the request of the Forest Biotechnology Committee the following condensed report on a questionnaire and Workshop organized by this committee is published as an addendum to these proceedings. The full length report is available from A. M. Stomp, North Carolina Biotechnology Center, Box 13547, Research Triangle Park, N.C. 27709-3547.

The committee has met once, on May 1, since the Workshop and adopted "Forest Biotechnology Committee" as its official name and agreed on a mission statement. Its activities will initially be focused on information exchange and the fostering of cooperation among various groups of scientists and users, including forest biotechnologists, forest tree breeders and forest physiologists.

VIEWS ON FOREST BIOTECHNOLOGY: REPORT FROM A CONFERENCE ON TREE IMPROVEMENT BY GENETIC ENGINEERING

A. M. Stomp[1/]
(Condensed by J. P. van Buijtenen)

INTRODUCTION

A three-day Workshop was held in North Carolina in January of 1987 to focus the thoughts and ideas of both scientists and industrial managers on the current technical state and future potential for tree improvement using genetic engineering approaches. Participants, from three principal interest groups, included government (USDA Forest Service and North Carolina Biotechnology Center), industry (timber, pulp and paper, and genetic engineering companies), and academic professionals from both the research and business management areas. Four goals were defined for the Workshop:

1) Assemble leading scientists, R&D directors and project managers for the purpose of establishing communication and initiating a framework for further cooperation in forest biotechnology R&D.

2) Define the present informational and policy constraints impeding immediate progress and efficient utilization of resources in the application of existing biotechnologies to current problems of forest tree use and biomass production.

3) Define existing opportunities for advancement in forest biotechnology.

4) Pinpoint areas where further basic research is required to maximize forest tree production and utilization.

[1/] Assistant Professor of Forestry, North Carolina State University, Raleigh, NC.

To accomplish these goals, the Workshop was structured with two parts. The first consisted of a questionnaire sent to research and management professionals to poll perceptions on the current state and potential value of genetic engineering to tree improvement. The second phase was the Workshop which assembled 30 participants from the principal interest groups to address the goals outlined above. This meeting was held at the Aqueduct in Chapel Hill, North Carolina under the joint sponsorship of the North Carolina Biotechnology Center, the United States Forest Service and the School of Forest Resources of North Carolina State University. What follows is the results of the questionnaire and the Workshop.

THE QUESTIONNAIRE

A questionnaire designed to poll current perspectives on the status and future direction of forest biotechnology was prepared and mailed to 138 professionals in the three interest groups. Following are the questions and a summary of the responses:

1) What is your perception of the current state of forest biotechnology? For example, what areas do you feel are developed or developing, e.g. clonal forestry, tissue culture, identification of early markers for superior trees, gene identification, gene transfer, etc.? What areas do you feel are not developed?

More than half of those responding felt that clonal forestry, i.e. multiplying selected material for operational planting through any vegetative method, is the most advanced technology. Methods cited for clonal forestry included rooted cuttings, micropropagation and tissue culture. A definite difference is perceived between propagation/plant regeneration methods for angiosperm vs gymnosperm species, with more difficulty encountered with gymnosperms. Both somatic embryogenesis and plant regeneration from small numbers of cells, e.g. shoot regeneration from callus, was cited as a critical need.

The general consensus was that DNA (gene) transfer was in its infancy but that method development was starting, e.g. the recent successful transfer of glyphosate resistance to hybrid poplar. About one third of those responding felt that much more work should be done to develop DNA transfer methods for important forest tree species, especially conifers.

Concern about a critical lack of information focused around four points: 1) Virtually no information exists on the physiological and molecular mechanisms of interesting phenotypes, e.g. drought resistance, disease resistance, wood characteristics. 2) Very little information exists about genomic structure, gene identification and mapping, and gene regulation for any forest tree species. 3) Mechanisms are not currently in place to integrate new biotechnology efforts with existing tree breeding work. This aspect was pinpointed as critical for long-term testing or genetically manipulating plants and for the development of biochemical markers which could be used for early screening of progeny. The

development of "early" markers was considered a top priority problem. 4) There is controversy over the characteristics which make a 'superior" tree. (Editor's note: Desirable traits will depend much on how the trees will be used).

2) What do you think are the most important processes and properties contributing to efficient productivity of forest trees? What do you think are the biological bottlenecks to increasing forest productivity and/or tree use?

The answers fell into two categories, abiotic and biotic stresses and limitations inherent in the trees themselves. Abiotic and biotic stresses were considered more important and were ranked as: disease resistance, nutrient utilization (especially nitrogen), and cold and water stress.

Overall, aspects of tree biology which may limit forest productivity were not considered as important as environmental limitations. Areas of interest included increasing photosynthetic efficiency, growth rate, wood quality, fiber length, other fiber characteristics, cellulose and lignin amounts and characteristics, and all aspects of canopy development including light interception, and root-shoot ratio. Other characteristics of interest included respiration, juvenile-mature transition and floral induction, pollination and fertilization as it pertains to both increased production of seed, identification of male steriles and total loss of reproductive capacity.

One frequently discussed item was genotype x environment interaction. Tailoring trees to site was thought as having significant potential for increasing stand productivity. Another idea frequently mentioned was developing the exploitative tree, one that was more environmentally opportunistic and thus could capitalize, through rapid growth, on favorable environmental conditions.

3) What are the most economically important forest tree species? Are there species which could serve as model systems for the development of technologies?

Selection of species was a regional issue, therefore no one species could be singled out as the most important. Coniferous species were considered more important than angiosperm species in the U.S., Canada, northern Europe and Central America. Loblolly pine (Pinus taeda) and Douglas fir (Pseudotsuga menziesii) are considered far more important than other coniferous species.

4) With respect to each of the important properties and processes you listed in question 1 above, what do you think are the best biotechnological approaches for increasing the efficiency of productivity? Why? For example, consider recombinant DNA techniques, the use of somaclonal variants, organelle transfer, clonal forestry, sexual tree breeding and

generation of molecular or cellular characteristics for early selection of superior individuals.

A unanimous view in favor of strong programs in conventional breeding was articulated. More work should be done on precocious flowering to accelerate breeding programs. The short-term gains from biotechnology are expected to come from some sort of clonal propagation of desirable phenotypes. Long-term gain is expected to come from recombinant DNA/gene transfer studies, but not through genetic engineering but through the molecular markers identified by this technology and the increase in understanding of the molecular mechanisms controlling tree phenotypes.

5) What processes and properties listed in question 1 do you think are most tractable for improving tree use or productivity? for example, consider herbicide resistance, insect and herbivore resistance, wood quality, tree growth rate, tree morphology, soil nutrient utilization, photosynthetic efficiency and novel tree use or secondary product production.

6) What do you think are the most rewarding/least risk/most sensible targets for increasing productivity in the short-term? mid-term? long-term?

Questions 5 & 6 dealt with what are the most important areas for research vs what are perceived as the most scientifically tractable projects for the short-, mid- and long-term. Virtually all responders agreed that the most important research effort should be in studies of tree physiology, biochemistry, cell and molecular biology and genetic structure and regulation with wood quality, phosynthetic efficiency, pest (disease, insect and herbivore) resistance and tree morphology receiving major emphasis. Although many were quick to acknowledge that genetically engineering herbicide resistance is feasible now and has been done in hybrid poplar, skepticism was voiced as to its value and economic justification. A large percentage of responders were concerned about the lack of good tissue culture methods for routine propagation of plants from single cells or cell clumps and for mass propagation of selected individuals of economically valuable species, especially conifers.

7) What sort of organization and strategy would you prefer to follow-up this Workshop, continue your involvement and maximize future cooperation of the principle interest groups in forest biotechnology?

Two suggestions received much attention, the creation of a newsletter and the creation of a new, broadly based organization. The organization would be a small one with representatives of government, wood and pulp & paper industries, genetic engineering concerns and academic and institute researchers to periodically discuss and review progress in the field and means to promote continued research. Other suggestions were to affiliate with

existing groups such as TAPPI, the Society of American Foresters and IUFRO working parties.

THE WORKSHOP

The Workshop assembled 30 participants from four major interest groups. They were asked to develop a strategy for the application of biotechnology to tree improvement which incorporated both scientific tractability and economic reality. The approach included the following tasks:

1) Prepare a prioritized list of economically important phenotypes for potential improvement by genetic engineering.

2) Determine the current extent of basic understanding of the molecular and genetic basis of economically important phenotypes.

3) Determine the current application of biotechnology to tree species.

4) Identify the critical technologies and information which are presently unavailable and necessary to apply genetic engineering to tree improvement.

Potential Traits for Genetic Engineering

Workshop discussion quickly focused on the fact that much of the future application of biotechnology to forestry will be determined by the perceived return on investment. In prioritizing a list of phenotypes for genetic improvement, difficulty arose in that it was impossible to determine cost or economic return on any phenotype. However, general guidelines on the cost side were given by Dr. Ron Sederoff, USDA-Forest Service, and on the return side by Dr. Pat Trotter, Weyerhaeuser Company.

Two factors determining the cost of manipulating a single gene were identified by Dr. Sederoff: 1) the amount of information that is known about the gene's identity at the molecular level and 2) the availability of methods for engineering in the selected species. A convenient way to express this is in scientist years, with the average cost of a scientist year averaging $100,000. If the gene product's identity, i.e. the enzyme or protein, is known and no new technology needs to be invented, the time required to isolate (clone) the gene will be about 2-3 years; to put that gene into a plant will be an additional 2-3 years. If molecular information is not available or if technologies, such as tissue culture methods or DNA transfer methods, need to be developed, the time required to move a gene into a plant becomes much longer. Extrapolating from the few examples that exist, such as herbicide resistance, a reasonable time period required before application would be 8-10 years.

Obtaining estimates on the return side of the investment equation is equally difficult. Several determining factors were identified by Dr. Trotter. The largest of these is the cost of getting the raw material to the mill gate. Genetic manipulation which lowers this cost could be valuable. Most of the value-added gain is accrued in processing the raw material,

therefore, genetic manipulation for quality or new products could be valuable. However, other factors play a role as well. When the raw material is plentiful, cost is low and market share is more important in determining profit. Under these conditions quality and product innovation are paramount. When wood is in short supply, bigger harvests are more important and faster growth rate giving higher yield increases profits. In either case, very large changes in returns, e.g. for the largest companies amounts on the order of $100 million, are required to attract investment. Clearly, there is no general formula for investment strategy.

The group's consensus of economically important phenotypes is given in Table 1. These characteristics have always been important factors in determining profitability and fall into two categories, "insurance" traits and "investment" traits. Insurance traits are those which protect trees from biotic and abiotic stresses, thereby preventing crop loss, i.e. increasing yield. Investment traits are those which alter the tree for specific processing or end-use, thereby increasing yield, value or creating a new product. Economic returns of all traits listed is dependent on environment.

Table 1.--Economically Important Traits.

Insurance Traits	Investment Traits
Biotic Stress	Wood Quality
Disease resistance	Chemistry
Insect resistance	Fiber length
Abiotic Stress	Growth Characteristics
Drought	Juvenile to mature transition
Heat & cold	Carbon allocation, harvest index
Air pollution, ozone	Morphology, apical dominance
Herbicide resistance	Growth Rate
	Nutrient Utilization

Requirements to Engineer These Traits

As outlined by Sederoff, the ability to manipulate any of the phenotypes listed in Table 1 will be proportional to our understanding of the genetic basis of the particular trait and the availability of methodologies to manipulate the trait at the molecular level. The group agreed that the largest need exists for information on the molecular basis and genetic regulation of economically important phenotypes. This need exists in agronomic crop species as well, however often less is known about tree species. Method development also lags. Two areas require immediate attention: 1) tissue culture regeneration for mass production of plants and 2) DNA transfer methods, with special focus on coniferous species.

Method Development

Method development in tree species generally lags far behind agronomic crops. Where Japanese breeding programs with Cryptomeria japonica are more

than 200 years old, conventional sexual breeding of forest tree species are less than 50 years old. In the oldest of these conventional U.S. programs, breeding is now approaching the third generation. Long breeding cycles and progeny testing coupled with the lack of breeding tools such as inbred lines, greatly limits the usefulness of conventional breeding programs to produce improved planting stock in a reasonable time scale. Therefore, tree improvement via genetic engineering has great potential if critical methods and information are forthcoming.

Key biotechnological methods critical for molecular investigations of genetic regulation are only beginning to be developed for use with tree species. Tissue culture methods are generally better developed for angiosperm tree species than gymnosperm species. A complete regeneration system from protoplasts to shoots exists for hybrid poplar. Somatic embryo systems are available for sweetgum, American elm, and Norway spruce. Shoot regeneration from callus exists in several angiosperm species including Betula sp., Populus sp. and Eucalyptus sp.

Critical to the application of biotechnology to trees is the transfer and expression of DNA. DNA transfer and expression has been demonstrated in hybrid poplar by the transfer of a gene giving tolerance to the herbicide glyphosate. Although actively being pursued in several laboratories, these key techniques have yet to be developed for gymnosperms.

In summary, methods for molecular investigations and genetic engineering are not generally available in forest tree species. The two most critical needs are tissue culture regeneration and DNA transfer, the latter being completely unavailable in coniferous species for the production of plants with new genes. Without these vital technologies, gene identification becomes more difficult and genetic engineering impossible.

Lack of Basic Information

Even with full method availability, a genetic engineering approach to tree improvement would be impossible due to the almost complete lack of molecular information about the genetic basis of economically important phenotypes. This point is reiterated both in the questionnaire responses and in Workshop discussions. It is generally felt that the pace of method development outstrips the increase in understanding. A positive correlation exists between methods and information; as methods become more universally available, the rate at which information is gathered becomes greater.

Currently, the molecular basis of two of the traits listed in Table 1 are known. Genes are available which confer resistance to certain herbicides. One such gene, Aro A, has been transferred to, and expressed in hybrid poplar. The group generally agreed that herbicide resistance was not a phenotype of major economic importance, and would therefore only merit limited investment. The other trait about which a considerable amount of molecular information is known is lignin biosynthesis. The economic importance of this trait could possibly justify the amount of research investment necessary to reach the level of understanding required for genetic engineering. Very little molecular information is available for the other traits listed in Table 1. Therefore, given the complete lack of molecular information on many economically important phenotypes, it is imperative that

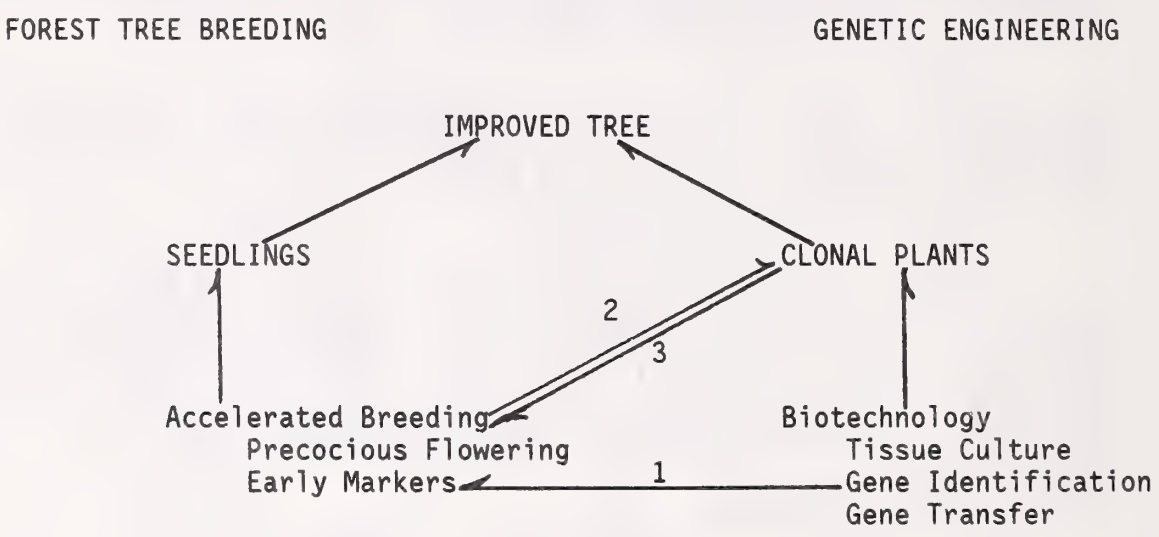
research immediately utilize existing tools to identify valuable genes.

STRATEGIC PLAN FOR THE APPLICATION OF BIOTECHNOLOGY TO TREE IMPROVEMENT

The group agreed that work to advance our abilities to genetically engineer trees needs to take place on two fronts: 1) continue to develop new and better methods to study the genetics of trees at the cellular and molecular level and 2) greatly increase the research effort on the genetic regulation of economically important traits.

The relationship between forest tree breeding and biotechnology is pictured in Figure 1.

Figure 1.--Relation between Forest Tree Breeding and Biotechnology.



Strategy

Workshop participants unanimously agreed that both conventional breeding and genetic engineering should be used for tree improvement. Presently, seedlings are used for reforestation. Parents are selected for general combining ability, therefore limiting the gain to the additive genetic component. It takes about 15 years for a seed orchard to reach full production and it takes at least 16 years to complete a breeding and testing cycle in loblolly pine. This cycle could be materially shortened if early markers related to future performance were available.

Biotechnology may hold promise for development of early markers (arrow 1). A key step in genetic engineering is the identification of genes regulating the phenotype of interest. Methods exist for screening individual organisms once the genes have been identified. Once a valuable gene has been identified and isolated (cloned), the next step in engineering an improved tree is to transfer that gene into a tissue culture regeneration system to

see if the gene is functioning as desired in the whole plant. Once this has been determined and any corrections made, tissue culture methods can again be used to regenerate large numbers of plants for operational plantings. Mass propagation methods could also be used to propagate the best individuals in the breeding population (clonal forestry), thus capturing both the additive and non-additive genetic components (arrow 2). Plants produced through genetic engineering could also be placed into the breeding population to move select genes into reforestation stock while maintaining broad genetic diversity of other traits (arrow 3).

Economic Impact of Biotechnology

The commitment to this research effort must be large and long-term. Is the potential economic gain from the application of biotechnology able to justify the long-term investment?

Table 2 gives the group's estimate of the potential increase in forest value and the time scale for application of the strategy for integrated tree improvement.

Table 2.--Gain Potential and Time Scale for Different Tree Improvement Techniques.

	Accelerated Breeding	Clonal Forestry	Gene Transfer
Potential Increase of forest Value above present	50%	40%	100%
Period of Application	present-long-term/	present-long-term/	mid-long-term/
Time before Application	currently available	available in short-term	available in long-term

* short-term: 3-5 years; mid-term: 6-10 years; long-term: 11-20 years

The potential impact of individual molecular and cellular techniques on the future success of tree improvement using conventional breeding, clonal forestry and gene transfer techniques can be examined as well. Table 3 estimates the magnitude of impact and the estimated time before specific technologies could be applied to the genetic manipulation of trees.

Another way to conceptualize the impact of biotechnology on tree improvement is through specific examples. For example, consider lignin biosynthesis. The lignin content of loblolly pine, a major pulp wood species, averages 28%. Lowering the lignin content to 23%, could result in a 10% increase in fiber yield. This increased yield would return $100 million annually to North Carolina pulp mills alone (H. M. Chang, personal communication). A way to look at the economic value of genetic gain would be to reduce it to the added value per acre (SAF Forestry Handbook and J. P. van

Buijtenen, personal communication). One percent genetic gain has been valued at $4.00/acre in 1987. If one assumes a minimum profit margin of 50%, then only half of the value of the genetic gain can be used to pay for its cost, or $2.00 per acre for 1% gain. dividing this by the average number of trees (loblolly pine) planted per acre, and adding this amount to $.03, a typical current price for bare-root loblolly pine seedlings, generates Table 4.

Table 3.--Impact and Time Scale of Specific Technologies.

Technique	Accelerated Breeding M/T*	Clonal Forestry M/T	Gene Transfer M/T
Markers/RFLP[1/]	high/short		medium/mid
Mapping	medium/mid		low/mid
Haploids	low/long		
Rejuvenation		high/mid	
Mass propagation		high/short	
Gene identification	medium/mid		high/mid-long
Vector Development			low/short
Somaclonal variants			low-medium/short
Organelle transfer			low/mid-long
Regeneration of transformed plants			high/short

* M:magnitude of the impact; T:time before techniques will be developed.
1/ RFLP: restriction fragment length polymorphisms.

Table 4.--Target Price of Propagules based on Estimated Genetic Gains.

Gain	Added value/acre	Target price/propagule @ 700 trees/acre
5%	$ 20	$ .045
10%	40	.06
25%	100	.09
50%	200	.17
100%	400	.31

CONCLUSIONS

The potential for using biotechnology to modify economically important genetic traits in trees is large and long-term. Both the questionnaire and the Workshop participants identified two categories of traits for modification: 1) "insurance" traits which protect against crop loss from biotic and abiotic stress, and 2) "investment" traits which alter the tree to lower production costs, and create better or new products. Modification of trees by genetic engineering requires information identifying the specific

molecular and cellular processes which determine the trait of interest and a sophisticated set of tools, called biotechnology, to manipulate the tree's biology. Both the basic information and the tools are critical; lack of either greatly inhibits or often prevents progress. Key tools requiring immediate development are plant regeneration from cells (tissue culture) and gene transfer methods, especially in coniferous species. In addition, we have virtually no knowledge of the molecular processes underlying the most important economic traits of trees. Research in basic molecular genetics of interesting traits is essential for future application of genetic engineering to tree improvement.

SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE
June 16-18, 1987
Registrants

Adams, John C.
Louisiana Tech University
School of Forestry
Ruston, Louisiana 71271

Apgar, Laurie J.
Virginia Dept. of Forestry
New Kent Forestry Center
Rt. 2, Box 42A
Providence Forge, VA 23140

Appel, David N.
Texas A&M University
Dept. of Plant Pathology
and Microbiology
College Station, Texas 77843

Arnold, Bill
Georgia Kraft Co.
P. O. Box 296
Greensboro, GA 30642

Askew, George R.
Research Forest Science Institute
P. O. Box 596
Clemson University
Georgetown, S.C. 29442

Balocchi, Claudio
E. S. King Village P-216
Raleigh, N.C. 27607

Barbour, Jill
Owens-Illinois, Inc.
P. O. Drawer A
White Springs, Florida 32096

Barnum, Garner
Arkansas Forestry Commission
P. O. Box 4523, Asher Station
Little Rock, Arkansas 72214

Bassala, Philippe
Texas A&I University
Campus Box 218
Kingsville, Texas 78363

Becwar, Michael R.
Institute of Paper Chemistry
P. O. Box 1039
Appleton, WI 54912

Beineke, Walter
Purdue University
Dept. of Forestry
and Natural Resources
West Lafayette, IN 47907

Bell, Wayne R.
International Forest Seed Co.
P. O. Box 290
Odenville, Alabama 35120

Belonger, Paul J.
Florida Division of Forestry
Rt. 1, Box 84
Milton, Florida 32570

Bilan, M. Victor
Stephen F. Austin State Univ.
SFASU Sta. Box 6109
Nacogdoches, Texas 75962

Blush, Tom
Westvaco
Forest Science Laboratory
Box 1950
Summerville, S.C. 29484

Bongarten, Bruce
University of Georgia
School of Forest Resources
Athens, Georgia 30602

Bosch, Leonard
Bosch Nursery
Rt. 2, Box 142A
Jonesboro, LA 71251

Bower, David R.
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, Arkansas 71901

Bower, Ralph C.
MacMillan Bloedel, LTD
65 Front Street
Nanaimo, British Columbia
V9R 5H9

Bramlett, David L.
U. S. Forest Service
Rt. 1, Box 182A
Dry Branch, Georgia 31020

Brantley, Franklin
Weyerhaeuser Co.
P. O. Box 187
Lyons, GA 30436

Bridgwater, Floyd
USDA Forest Service
NCSU Box 8002
Raleigh, NC 27695-8002

Brooks, Terrell L.
Georgia Forestry Commission
Rt. 1, Box 183
Dry Branch, Georgia 31020

Brown, George, Jr.
Alabama A&M University
P. O. Box 183
Dept. of Plant & Soil Science
Normal, Alabama 35762

Brown, I. N., Jr.
Texas Forest Service
Rt. 5, Box 109
Kirbyville, Texas 75956

Bryant, Richard
International Paper Company
Rt. 1, Box 571
Bainbridge, Georgia 31717

Burris, Leon
Weyerhaeuser Company
900 Whittington Ave.
Hot Springs, Arkansas 71901

Byram, Tom D.
Texas Forest Service
Forest Science Laboratory
College Station, Texas 77843

Cameron, Scott
Texas Forest Service
P. O. Box 310
Lufkin, Texas 75901

Canavera, Dave
Westvaco
Box 1950
Summerville, S.C. 29484

Carroll, Lee
Champion International
P. O. Box 359
Livingston, Texas 77351

Chambers, Jim L.
Louisiana State University
School of Forestry, Wildlife
and Fisheries
Baton Rouge, Louisiana 70803

Chandler, Charles R.
Texas Forest Service
P. O. Box 853
Rusk, Texas 75785

Chase, Charles
St. Joe Land & Development Co.
Rt. 1, Box 70
Lamont, Florida 32336

Chavan, S. A.
School of Natural Resources
2021 Coffey Road, 367C, KH
Columbus, Ohio 43210-1085

Clark, Jake
Catawba Timber Company
P. O. Box 128
Catawba, S. C. 29730

Coleman, Stephen
Boise Cascade Company
P. O. Box 37
Singer, Louisiana 70660

Cunningham, Mike
N. C. State University,
Forestry Dept.
Hardwood Research Cooperative
NCSU Box 8002
Raleigh, N.C. 27695-8002

Dalrymple, Debbi
Weyerhaeuser Company
Rt. 5, Box 250
Magnolia, Arkansas 71753

Davison, Marc
Champion International
P. O. Box 97
Tillery, N.C. 27887

DeBarr, Gary L.
U. S. Forest Service
Forestry Sciences Laboratory
Carlton Street
Athens, Georgia 30602

DeWald, Laura
Univ. of Minnesota
110 Green Hall
Dept. of Forest Resources
St. Paul, MN 55108

Dinus, Ronald J.
Institute of Paper Chemistry
P. O. Box 1039
Appleton, WI 54912

Du, Wei
Dept. of Forest Resources
University of Idaho
Moscow, Idaho 83843

Emadian, Seyed F.
Forest Genetics Laboratory
Texas A&M University
College Station, Texas 77843

Felker, Peter
Texas A&I University
Campus Box 218
Kingsville, Texas 78363

Feret, Peter
Virginia Tech
Dept. of Forestry
Blacksburg, VA 29060

Forward, Paul
Forest Service - USDA
RFD #4, N. Marigold CT
Warrenton, VA 22186

Foster, Sam
International Forest Seed Co.
P. O. Box 290
Odenville, Alabama 35120

Francis, Bruce
Weyerhaeuser Company
Tree Improvement Facility
P. O. Box 1060
Hot Springs, Arkansas 71902

Gates, James
USDA - Forest Service
2500 Shreveport Highway
Pineville, Louisiana 71360

Gerwig, Davis M.
Westvaco
P. O. Box 1950
Summerville, S. C. 29484

Gladstone, William T.
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, Arkansas 71902

Glumac, Edward
University of Houston
Biology Dept.
Houston, TX 77004

Govindaraju, D. R.
Dept. of Forestry
University of Kentucky
Lexington, KY 40546

Greene, Thomas A.
Texas Forest Service
Forest Science Laboratory
College Station, Texas 77843

Guinness, Bill
Catawba Timber Company
P. O. Box 128
Catawba, S.C. 29704

Haines, L. Wayne
International Paper Co.
Rt. 1, Box 571
Bainbridge, GA 31717

Hall, Oscar
USDA - Forest Service
Seed Lab
Rt. 1, Box 182-B
Dry Branch, GA 31020

Hall, Richard B.
Iowa State University
Dept. of Forestry
Ames, Iowa 50011

Hallgren, S. W.
Dept. of Forestry
Oklahoma State University
Stillwater, Oklahoma 74078

Hannah, Wayne
Hannah's
Rt. 3, Box 20-B
Kirbyville, Texas 75956

Hanover, James W.
Michigan State University
Department of Forestry
E. Lansing, MI 48824

Harbin, Michael
Chesapeak Corporation
Box 311
West Point, VA 23181

Hart, E. R.
Iowa State University
Dept. of Entomology
Ames, Iowa 50011

Hatcher, Alice
N. C. State Univ.
Cooperative Tree
Improvement Program
NCSU Box 8002
Raleigh, N.C. 27695

Heard, Jimmy
Willamette Industries, Inc.
General Delivery
Taylor, Louisiana 71080

Hendrickson, John A.
Scott Paper Company
P. O. Box 899
Saraland, Alabama 36571

Herr, Glen
Boise Cascade Company
P. O. Box 37
Singer, Louisiana 70660

Hicks, Van Joseph, Jr.
Dept. of Agriculture & Forestry
P. O. Box 337
DeRidder, Louisiana 70634

Hodge, Gary
CFGRP, Univ. of Florida
118 N-Z Hall
Gainesville, Florida 32611

Hodges, James
Brunswick Pulp Land Co.
Box 860
Brunswick, GA 31521

Hollowell, A. R.
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, Arkansas 71902

Hook, Don
Clemson University
Forest Science Lab
2730 Savannah Highway
Charleston, S.C. 29407

Huang, Feng Hou
University of Arkansas
316 Plant Science Bldg.
Fayetteville, Arkansas 72701

Huber, Dudley
University of Georgia
School of Forest Resources
Athens, Georgia 30602

Hughes, Henry
International Paper Company
Rt. 1, Box 571
Bainbridge, Georgia 31717

Hunt, Jeanne L.
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, Arkansas 71902

Jacobs, Bill
Champion International
P. O. Box 359
Livingston, Texas 77351

Jain, Shri Mohan
Texas A&M University
Dept. of Forest Science
College Station, Texas 77843

Jambulingam, R.
School of Forestry Resources
N. C. State University
NCSU Box 8002
Raleigh, N.C. 27695

Janssen, Jill
Texas A&M Univ.
Dept. of Forest Science
College Station, Texas 77843

Jett, J. B.
N. C. State University
NCSU Box 8002
Raleigh, N.C. 27695

Jones, Barbara
Weyerhaeuser Company
Tree Improvement Facility
P. O. Box 1060
Hot Springs, Arkansas 71902

Khajuria, H. N.
NCSU School of Forest Res.
Dept. of Forestry
Raleigh, N.C. 27695

Kraft, Patrick
Mississippi Forestry Comm.
301 N. Lamar, Suite 300
Jackson, Mississippi 39201

Kucera, R. (Bob)
Alabama Forestry Commission
513 Madison Ave.
Montgomery, Alabama 36130

Kung, Fan H.
Southern Illinois University
Dept. of Forestry
Carbondale, Illinois 62901

LaFarge, Timothy
USDA - Forest Service
1720 Peachtree Rd., NW
Atlanta, Georgia 30367

Land, Samuel B. Jr.
Mississippi State University
P. O. Drawer FR
Mississippi State, MS 39762

Lantz, Clark W.
U.S. Forest Service
Suite 811 N
1720 Peachtree Road, N.W.
Atlanta, Georgia 30367

Larson, Dale R.
Gulf States Paper Corporation
P. O. Box 3199
Tuscaloosa, Alabama 35404

Layton, Pat
Oak Ridge National Lab
P. O. Box Y
Oak Ridge, TN 37931

Leach, Gregory
Champion International
P. O. Box 875
Cantonment, FL 32533

Lee, Joel T.
Texas A&M University
Forest Science Dept.
College Station, Texas 77843

Li, Bailian
N. C. State University
NCSU Box 8002
Raleigh, N. C. 27605

Long, Ernest M.
International Paper Company
P. O. Box 2220
Nacogdoches, TX 75963

Loveless, Robert W.
Container Corporation of America
P. O. Box 1469
Brewton, Alabama 36426

Lowe, William J.
Texas Forest Service
Forest Science Laboratory
College Station, Texas 77843

Lowerts, George A.
Union Camp Company
P. O. Box 216
Rincon, GA 31326

Lyons, Al
Kimberly-Clark Corporation
Forest Management Office
Coosa Pines, Alabama 35044

Mahalovich, Mary F.
N.C. State University
Department of Forestry
NCSU Box 8002
Raleigh, N.C. 27695

Marco, Martin A.
Univ. of Florida
Dept. of Forestry
118 Newins-Ziegler Hall
Gainesville, Florida 32611

Marty, Trenten
Wisconsin Dept. of Natural
Resources
Box 7921
Madison, WI 53707

Massie, Bill
Mississippi Forestry Comm.
P. O. Box 468
Lumberton, Mississippi 39455

Maynor, Maxie
Federal Paper Board
P. O. Box 1007
Lumberton, N. C. 28359

McCall, Early Y.
ITT Rayonier, Inc.
Morgan Research Center
P. O. Box 437
Yulee, Florida 32097

McConnell, James L.
U. S. Forest Service
1720 Peachtree Rd., Suite 816
Atlanta, Georgia 30367

McCullough, Rex B.
Weyerhaeuser Company
WTC-2H2
Tocoma, WA 98477

McGriff, James A.
Georgia-Pacific Corporation
P. O. Box 520
Crossett, Arkansas 71635

McKeand, Steven E.
NCSU Tree Improvement
Cooperative
NCSU Box 8002
Raleigh, N.C. 27695

McKinley, Craig R.
Texas Forest Service
Forest Science Dept.
College Station, Texas 77843

McRae, John
International Forest Seed Co.
P. O. Box 290
Odenville, Alabama 35120

Meier, C. E.
Forest Science Dept.
Texas A&M University
College Station, Texas 77843

Miller, Dot
Weyerhaeuser Company
900 Whitting Ave.
Hot Springs, Arkansas 71902

Miller, Larry
Temple-Eastex Forests
229 N. Bowie
Jasper, Texas 75951

Miller, Thomas
U. S. Forest Service
Dept. of Forestry
Univerisity of Florida
Gainesville, Florida 32611

Morrow, Daniel F.
Maryland Park and Wildlife
Services
Buckingham Forest Tree Nursery
Harmans, MD 21077

Mukewar, A. M.
P. O. Drawer FR, MSU
Mississippi State, MS 39762

Murillo, Olman
1712-C Collegeview Ave.
Raleigh, N.C. 27606

Nance, Warren L.
USDA-Forest Service
Southern Forest Experiment St
P. O. Box 2008, GMF
Gulfport, MS 39505

Newton, Ron
Forest Science Dept.
Texas A&M University
College Station, Texas 77843

Nyong'o, Risper
Iowa State University
Dept. of Forestry
Ames, Iowa 50011

Oak, Steven W.
USDA Forest Service
Southern Region FPM
Rt. 3, Box 1249 A
Asheville, N.C. 28806

Patil, C. S. P.
P. O. Drawer FR
Mississippi State, MS 39762

Pohl, Russell
Georgia Forestry Commission
1843 Kirkwood Drive
Macon, Georgia 31211

Powell, Gregory
Univ. of Florida
118 Newins-Ziegler Hall
Gainesville, Florida 32611

Powers, Harry R.
U. S. Forest Service
Forestry Sciences Lab
Carlton Street
Athens, GA 30605

Rang, Allah
Dept. of Forestry
Michigan State University
East Lansing, MI 48824-1222

Robinson, John F.
Texas Forest Service
Hort. For. Sci. Bldg.
College Station, Texas 77843

Rousseau, Randall J.
Westvaco
Box 458
Wickliffe, KY 42087

Rucker, Terry
Weyerhaeuser Company
Rt. 3, Box 365
Washington, N.C 27889

Schmidtling, Ron
U. S. Forest Service
P. O. Box 2008 GMF
Gulfport, MS 39505

Schalchli, Alejandra
E. S. King Village P-216
Raleigh, N. C. 27607

Sehgal, Ravinder N.
Dept. of Forestry
Michigan State University
East Lansing, MI 48824

Shear, Ted
Dept. of Forestry
Clemson University
2730 Savannah Hwy.
Charleston, S. C. 29407

Smith, W. Rick
USDA Forest Service
255 Cherokee Street
New Orleans, LA 70118

Snow, Glenn
USDA Forest Service
BOX 2008 GMF
Gulfport, MS 39505

Sommer, Harry E.
School of Forest Resources
University of Georgia
Athens, Georgia 30602

South, David
Nursery Co-op
School of Forestry
Auburn University
Auburn, Alabama 36849

Souza, Sonia M.
University of Florida
School of Forestry
118 Newins-Zeigler Hall
Gainesville, FL 32611

Sprinz, Peter
Texas A&M University
Dept. of Forest Science
College Station, Texas 77843

Stauder, Albert
Texas Forest Service
P. O. Box 617
Alto, Texas 75925

Steele, Jim
Temple-Eastex Forests
229 N. Bowie
Jasper, Texas 75951

Steigerwalt, Mark
Continental Forest
Investments, Inc.
P. O. Box 8969
Savannah, Georgia 31412

Steiner, Kim
Penn State University
Forest Resources Lab
University Park, PA 16802

Stelzer, Hank
University of Florida
118 N-Z Hall
Dept. of Forestry
Gainesville, Florida 32611

Strub, Mike
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, Arkansas 71902

Stubbs, Olin L.
Dept. of Agriculture
and Forestry
P. O. Box 1628
Baton Rouge, Louisiana 70821

Summerville, K. O.
N. C. Division of Forest
Resources
2411 Garner Road
Clayton, N.C. 27520

Sun, Zhongxu
University of Kentucky
Department of Forestry
Lexington, KY 40546

Tang, Zhemming
Mississippi State University
P. O. Drawer FR
Mississippi State, MS 39762

Tauer, C. G.
Oklahoma State University
012 Ag. Hall, Dept. of Forestry
Stillwater, Oklahoma 74078

Teclaw, Ron
U. S. Forest Service
P. O. Box 898
Rhinelander, WI 54501

Teskey, Robert
University of Georgia
School of Forest Resources
Athens, Georgia 30602

Tewari, Salil K.
Ohio State University
School of Natural Resources
2021 Coffey Road
Columbus, Ohio 43210

Todhunter, Mike
International Paper Company
Rt. 1, Box 571
Bainbridge, Georgia 31717

Toliver, John
Louisiana State University
School of Forestry, Wildlife
and Fisheries
Baton Rouge, Louisiana 70803

Tuskan, Jerry
North Dakota State University
Fargo, ND 58109

Valluri, Jagannath
Texas A&M University
Forest Science Dept.
College Station, TX 77843

van Buijtenen, J. P.
Texas Forest Service
Forest Genetics Laboratory
College Station, Texas 77843

Walkinshaw, Camille
USDA - Forest Service
P. O. Box 2008 GMF
Gulfport, Mississippi 39505

Walkinshaw, Charles
USDA - Forest Service
P. O. Box 2008 GMF
Gulfport, Mississippi 39505

Waxler, Mike
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, Arkansas 71902

Weir, Robert J.
NCSU-Cooperative Tree
Improvement Program
NCSU Box 8002
Raleigh, N.C. 27695

White, Tim
Dept. of Forestry
Univ. of Florida
118 N-Z Hall
Gainesville, Florida 32611

Williams, Claire
Duke University
School of Forestry
Durhan, N.C. 27607

Williford, Mike
Hiwassee Land Co.
Rt. 4, Box 211
Chatsworth, GA 30705

Windham, Jerry
USDA-Forest Service
Rt. 1, Box 621
Brooklyn, MS 39425

Wright, Jeff
Sappi Forests
P. O. Box 75
Pietermaritzoburg 3200
South Africa

Yates, Harry O. III
USDA-Forest Service
Forestry Sciences Laboratory
Carlton Street
Athens, GA 30602

Yi, Jae-Seon
University of Georgia
School of For. Resources
Athens, Georgia 30602

Zoerb, Marvin
Union Camp Corp.
P. O. 216
Rincon, GA 31326

SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

Membership List

June 18, 1987

Group A

Representatives Appointed for 6-year term

Committee Membership	Term Ends (at end of Annual meeting)	Representing
E. C. Burkhardt 5423 Fisher Ferry Rd. Vicksburg, MS 39180 (601)636-1045	1989	Hardwood Research Council
Marvin Zoerb Union Camp Corp. P. O. Box 216 Rincon, GA 31326 (912)826-5556 Chairman	1989	Forest Industry
Siroos T. Jahromi International Paper Co. Southlands Experiment Forest Rt. 1, Box 571 Bainbridge, GA 31717 (912)246-3642 Secretary/Treasurer	1992	Forest Industry
Mike Waxler Southern Operations Geneticist Weyerhaeuser Corporation P. O. Box 1060 Hot Springs, AR 71902 (501)624-8494	1992	Forest Industry
Garner Barnum Management Forester Arkansas Forestry Commission P. O. Box 4523, Asher Station 3821 W. Rosevelt Road Little Rock, AR 72214 (501)664-2531	1988	State Forestry Agencies
Bob Kucera Alabama Forestry Commission 513 Madison Avenue Montgomery, AL 36130-0601 (205)261-2540	1988	State Forestry Agencies

Russell Pohl Georgia Forestry Commisssion Box 819 Macon, GA 31298-4599 (912)774-3354	1992	State Forestry Agencies
Sam B. Land, Jr. Dept. of Forestry Mississippi State University P. O. Drawer FR Mississippi State, MS 39762 (601)325-2946	1988	Forestry Schools
Charles Tauer 08 AG Hall Oklahoma State University Stillwater, OK 74074 (405)624-5462	1989	Forestry Schools
John Toliver School of Forestry and Wildlife Management Louisiana State University Baton Rouge, LA 70803 (504)388-4131	1992	Forestry Schools
James Hodges Research and Resource Division Brunswick Pulp Land Company P. O. Box 860 Brunswick, GA 31520 (912)265-5780	1992	Forest Industry

GROUP B

Representatives Appointed for Indefinite Terms

Floyd Bridgwater USDA Forest Service Southeastern Forest Expt. Sta. School of Forest Resources Box 8002 N. C. State University Raleigh, NC 27695-8002 (919)737-3168	U. S. Forest Service Southeastern Forest Expt. Sta.
Clark Lantz Cooperative Forestry U. S. Forest Service Suite 811 1720 Peachtree Road, N.W. Atlanta, GA 30367 (404)881-3551	U. S. Forest Service State & Private Forestry

Warren Nance
Institute of Forest Genetics
P. O. Box 2008, GMF
Gulfport, MS 39503
(601)864-3972

U. S. Forest Service
Southern Forest
Expt. Station

GROUP C

Specialists Appointed for 6-year Term

Ralph Mott Botany Dept. North Carolina State Univ. Box 7612 Raleigh, NC 27695-7612 (919)737-3525	1990	Forest Biotechnology
Warren Nance Institute of Forest Genetics Box 2008, GMF Gulfport, MS 39503 (601)864-3972	1990	Stand Dynamics
Harry O. Yates Southeastern For. Expt. Sta. Carlton Street Athens, GA 30601 (404)546-2476	1990	Entomology
Ron Schmidtling Institute of Forest Genetics Box 2008, GMF Gulfport, MS 39503 (601)864-3972	1992	Racial Variation and Seed Movement
David Bramlett Southeastern Forest. Exp. Sta. Rt. 1, Box 182A Dry Branch, GA 31020 (912)744-0261	1993	Pollen Management
Tom Miller, IPM School of Forest Resources University of Florida Gainesville, FL 32611 (904)392-4826	1993	Pathology

GROUP D

Representatives Appointed for Indefinite Terms

James L. McConnell U. S. Forest Service Suite 828 1720 Peachtree Road, N.W. Atlanta, GA 30309 (404)881-3846	U. S. Forest Service Regional Office Region 8
J. P. van Buijtenen Forest Genetics Lab College Station, TX 77843 (409)845-1325	Western Gulf Forest Tree Improvement Cooperative
Bob Weir Forestry Department Box 8002 Raleigh, NC 27695-8002 (919)737-3168 Vice-Chairman	North Carolina State Tree Improvement Cooperative
Tim White School of Forest Resources and Conservation University of Florida Gainesville, FL 32611 (904)392-1850	University of Florida Tree Improvement Cooperative